THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant(s):	Nongnuch Inpanbutr
Appl. No.:	09/804,111
Conf. No.:	8670
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Title:	METHOD AND PRODUCT FOR TREATING CANCER IN PETS
Art Unit:	1617
Examiner:	Hui, San Ming
Docket No.:	115808-457

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPELLANT'S APPEAL BRIEF

Sir:

Appellant submits this Appeal Brief in support of the Notice of Appeal filed on February 24, 2005. This Appeal is taken from the Final Rejection in the Office Action dated August 26, 2004.

I. REAL PARTY IN INTEREST

The real party in interest for the above-identified patent application on Appeal is Nestec, Ltd. by virtue of an Assignment dated January 28, 2003 and recorded at reel 013388, frame 0006 in the United States Patent and Trademark Office.

II. RELATED APPEALS AND INTERFERENCES

Appellant's legal representative and the Assignee of the above-identified patent application do not know of any prior or pending appeals, interferences or judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision with respect to the above-identified Appeal.

III. STATUS OF CLAIMS

Claims 1, 3-6, 8-12, 18-26, 28-37 and 39-40 are pending in the above-identified patent application. Claims 2, 7, 13-17, 27 and 38 have been canceled previously. Claims 1, 3-6, 8-12, 18-26, 28-37 and 39-40 stand rejected. Therefore, Claims 1, 3-6, 8-12, 18-26, 28-37 and 39-40 40 are being appealed in this Brief. A copy of the appealed claims is included in the Claims Appendix.

IV. STATUS OF AMENDMENTS

No amendments were made in this application after the Final Rejection in the Office Action dated August 26, 2004.

V. SUMMARY OF CLAIMED SUBJECT MATTER

A summary of the invention by way of reference to the drawings and specification for each of the independent claims is provided as follows:

Independent Claim 1 is directed to a method of treating SCC 2/88, a canine squamous carcinoma cell line, for cancer, comprising the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic agent comprising a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof (page 4, lines 13-17 and page 8, lines 3-31).

Dependent Claim 6 is directed to the method of Claim 1, wherein the vitamin D analog is administered in combination with a bone agent, a cytotoxic agent, an immuno response regulating agent, an anti-inflammatory agent or combinations thereof (page 10, lines 3-31).

Dependent Claim 8 is directed to the method of Claim 1, wherein the dog is fed from about 0.025 to about 500 nmol/kg of body weight of the patient per day of the vitamin D analog (page 9, lines 1-6).

Dependent Claim 9 is directed to the method of Claim 1, wherein the dog is fed from about 0.025 to about 100 nmol/kg of body weight of the patient per day of the vitamin D analog (page 9, lines 1-6).

Dependent Claim 10 is directed to the method of Claim 1, wherein the dog is fed from about 0.025 to about 10 nmol/kg of body weight of the patient per day of the vitamin D analog (page 9, lines 1-6).

Dependent Claim 11 is directed to the method of Claim 1, wherein the dog is fed from about 0.025 to about 1.0 nmol/kg of body weight of the patient per day of the vitamin D analog (page 9, lines 1-6).

Dependent Claim 18 is directed to the method of Claim 1, wherein the Vitamin D analog is administered in combination with a bone agent comprising at least one of conjugated estrogens, conjugated estrogen equivalents, anti-estrogens, calcitonin, bisphosphonates, calcium supplements, calcium receptor agonists, cobalamin, pertusssis toxin, boron, dehydroepiandrosterone, activin and bone morphogenic protein (page 9, lines 3-9).

Dependent Claim 19 is directed to the method of Claim 1, wherein the Vitamin D analog is administered in combination with a cytotoxic agent comprising at least one of estramustine phosphate, prednimustine, cisplatin, 5-fluoro-uracil, melphalan, hydroxyurea, mitomycin, idarubicin, methotrexate, adriamycin, daunomycin, cyclophosphamide, doxorubicin, vincristine and pregnisone (page 9, lines 10-14).

Dependent Claim 20 is directed to the method of Claim 1, wherein the Vitamin D analog is administered in combination with an anti-inflammatory agent comprising at least one of a steroidal anti-inflammatory agent and a non-steroidal anti-inflammatory agent (page 9, lines 25-31).

Dependent Claim 21 is directed to the method of Claim 20, wherein the steroidal anti-inflammatory agent includes corticosteroids (page 9, lines 25-31).

Dependent Claim 22 is directed to the method of Claim 20, wherein the non-steroidal anti-inflammatory agent includes at least one of salicylates and naproxen (page 9, lines 25-31).

Dependent Claim 23 is directed to the method of Claim 1, wherein feeding the dog a therapeutic agent comprising a vitamin D analog comprises producing a pharmaceutical agent from admixture which includes at least one of a pharmaceutically acceptable organic carrier substance and a pharmaceutically acceptable inorganic carrier substance (page 8, lines 15-31).

Dependent Claim 24 is directed to the method of Claim 23, wherein the pharmaceutically acceptable organic carrier substance and the pharmaceutically acceptable inorganic carrier substance include at least one of water, salt and buffer solutions, alcohols, gum arabic, mineral and vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylase or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxyl methylcellulose, and polyvinyl pyrrolidine (page 8, lines 15-25).

Dependent Claim 25 is directed to the method of Claim 23, further comprising mixing the pharmaceutical agent with an auxiliary agent, the auxiliary agent including one or more of lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic active compounds (page 8, lines 25-31).

Independent Claim 26 is directed to a method of providing a therapeutic agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3

and stereoisomers thereof, said method comprising providing a pet food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet (page 4, lines 13-17 and page 10, lines 10-24).

Dependent Claim 28 is directed to the method of Claim 26, wherein the vitamin D analog is administered in combination with at least one of a bone agent, a cytotoxic agent, and immuno response regulating agent, and an anti-inflammatory agent (page 10, lines 3-31).

Dependent Claim 29 is directed to the method of Claim 26, wherein the dog is fed from about 0.025 to about 500 nmol/kg of body weight of the dog per day of the vitamin D analog (page 9, lines 1-6).

Dependent Claim 30 is directed to the method of Claim 26, wherein the dog is fed from about 0.025 to about 100 nmol/kg of body weight of the dog per day of the vitamin D analog (page 9, lines 1-6).

Dependent Claim 31 is directed to the method of Claim 26, wherein the dog is fed from about 0.025 to about 10 nmol/kg of body weight of the dog per day of the vitamin D analog (page 9, lines 1-6).

Dependent Claim 32 is directed to the method of Claim 26, wherein the dog is fed from about 0.025 to about 1.0 nmol/kg of body weight of the dog per day of the vitamin D analog (page 9, lines 1-6).

Independent Claim 34 is directed to a method of administering a pharmaceutical agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of $1\alpha,25\text{-}(OH)_2D_3$, $1\alpha,25\text{-}(OH)_2\text{-}16\text{-ene-}23\text{-yne-}D_3$ and $1\alpha,25\text{-}(OH)_2\text{-}22,24\text{-diene-}24,26,27\text{-trihomo-}D_3$ and stereoisomers thereof, said method comprising providing a pet food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet (page 4, lines 13-17 and page 8, lines 3-31).

Dependent Claim 35 is directed to the method of Claim 34 wherein feeding the pet food to a pet comprises producing a pharmaceutical agent from an admixture which includes at least one of a pharmaceutically acceptable organic carrier substance and a pharmaceutically acceptable inorganic carrier substance (page 8, lines 15-31).

Dependent Claim 36 is directed to the method of Claim 35 wherein the pharmaceutically acceptable organic carrier substance and the pharmaceutically acceptable inorganic carrier substance include at least one of water, salt (buffer) solutions, alcohols, gum arabic, mineral and

vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylase or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxyl methyl cellulose, and polyvinyl pyrrolidone (page 8, lines 15-25).

Dependent Claim 37 is directed to the method of Claim 35 further comprising mixing the pharmaceutical agent with an auxiliary agent that includes one or more of lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic active compounds (page 8, lines 25-31).

Dependent Claim 39 is directed to the method of Claim 34 wherein the dog is fed from about 0.025 to about 500 nmol/kg of body weight of the dog per day of the vitamin D analog (page 9, lines 1-6).

Although specification citations are given in accordance with C.F.R. 1.192(c), these reference numerals and citations are merely examples of where support may be found in the specification for the terms used in this section of the Brief. There is no intention to suggest in any way that the terms of the claims are limited to the examples in the specification. As demonstrated by the citations below, the claims are fully supported by the specification as required by law. However, it is improper under the law to read limitations from the specification into the claims. Pointing out specification support for the claim terminology as is done here to comply with rule 1.192(c) does not in any way limit the scope of the claims to those examples from which they find support. Nor does this exercise provide a mechanism for circumventing the law precluding reading limitations into the claims from the specification. In short, the specification citations are not to be construed as claim limitations or in any way used to limit the scope of the claims.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Claims 1, 3-5, 8-12, 23-26, 28-37 and 39-40 stand rejected under 35 U.S.C. §103(a) as unpatentable over U.S. Patent No. 5,087,619 to Baggiolini et al. ("*Baggiolini*") and Cancer Research, 1999; 59: 3325-3328 by Abdaimi et al. ("*Abdaimi*"). A copy of the Final Office Action dated 8/26/04 is attached as Exhibit A. Copies of *Baggiolini* and *Abdaimi* are attached herewith as Exhibits B and C, respectively.

2. Claims 6 and 18-22 stand rejected under 35 U.S.C. §103(a) as unpatentable over *Baggiolini* and *Abdaimi* as applied to Claims 1, 3-5 and 8-12 and in further view of Basic and Clinical Pharmacology, 1995, p. 537-538, 661-663, 830-832, 838 and 841 by Katzung ("*Katzung*") and Goodman and Gilman's The Pharmacological Basis of Therapeutics, 1996, p. 539 by Hardman et al. ("*Hardman*"). Copies of *Katzung* and *Hardman* are attached herewith as Exhibits D and E, respectively.

VII. GROUPING OF THE CLAIMS

Appellant argues for the separate patentability of each of the independent claims separate and apart from each other set forth in detail below pursuant to the requirements of 37 C.F.R. § 1.192(7), unless otherwise specified. In addition, Appellant argues for the separate patentability of certain of the dependent claims separate and apart from the claims from which they depend as noted below.

VIII. ARGUMENT

A. LEGAL STANDARDS

The Federal Circuit has held that the legal determination of an obviousness rejection under 35 U.S.C. § 103 is:

> whether the claimed invention as a whole would have been obvious to a person of ordinary skill in the art at the time the invention was made...The foundational facts for the prima facie case of obviousness are: (1) the scope and content of the prior art; (2) the difference between the prior art and the claimed invention; and (3) the level of ordinary skill in the art...Moreover, objective indicia such as commercial success and long felt need are relevant to the determination of obviousness...Thus, each obviousness determination rests on its own facts.

In re Mayne, 41 U.S.P.Q. 2d 1451, 1453 (Fed. Cir. 1997).

In making this determination, the Patent Office has the initial burden of proving a *prima facie* case of obviousness. *In re Rijckaert*, 9 F.3d 1531, 1532, 28 U.S.P.Q. 2d 1955, 1956 (Fed. Cir. 1993). This burden may only be overcome "by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings." *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q. 2d 1596, 1598 (Fed. Cir. 1988). "If the examination at the initial stage does not produce a prima facie case of unpatentability, then without more the applicant is entitled to grant of the patent." *In re Oetiker*, 24 U.S.P.Q. 2d 1443, 1444 (Fed. Cir. 1992).

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the reference or references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *In re Fine*, 837 F.2d 1071, 5, U.S.P.Q.2d 1596 (Fed. Cir. 1988). Second there must be a reasonable expectation of success. *In re Merck & Co., Inc.*, 800 F.2d 1091, 231 U.S.P.Q. 375 (Fed. Cir. 1986) Finally, all of the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q., 580 (CCPA 1974).

Further, the Federal Circuit has held that it is "impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." *In re Fritch*, 23 U.S.P.Q.2d 1780, 1784 (Fed. Cir.

1992). "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention" *In re Fine*, 837 F.2d 1071 (Fed. Cir. 1988).

Moreover, the Federal Circuit has held that "obvious to try" is not the proper standard under 35 U.S.C. §103. *Ex parte Goldgaber*, 41 U.S.P.Q.2d 1172, 1177 (Fed. Cir. 1996). "An-obvious-to-try situation exists when a general disclosure may pique the scientist curiosity, such that further investigation might be done as a result of the disclosure, but the disclosure itself does not contain a sufficient teaching of how to obtain the desired result, or that the claim result would be obtained if certain directions were pursued." *In re Eli Lilly and Co.*, 14 U.S.P.Q.2d 1741, 1743 (Fed. Cir. 1990).

B. THE REJECTION OF CLAIMS 1, 3-5, 8-12, 23-26, 28-37 AND 39-40 UNDER 35 U.S.C. §103(a) TO *BAGGIOLINI* AND *ABDAIMI* SHOULD BE REVERSED BECAUSE THE PATENT OFFICE HAS NOT ESTABLISHED A *PRIMA FACIE* CASE OF OBVIOUSNESS

1. The Cited References

Appellant respectfully submits that the obviousness rejection of Claims 1, 3-5, 8-12, 23-26, 28-37 and 39-40 should be reversed because the Patent Office fails to establish a *prima facie* case of obviousness. Regarding Claims 1, 3-5, 8-12, 23-26, 28-37 and 39-40, the Patent Office in the Final Office Action dated 8/26/2004 ("Office Action") at page 3 alleges that the combination of *Baggiolini* and *Abdaimi* renders obvious the claimed invention. However, the Patent Office fails to establish a *prima facie* case of obviousness in each rejection because there is no teaching or suggestion within the references cited or within the general knowledge of those skilled in the art that would have led one skilled in the art to make the combination suggested and the cited references fail to teach or suggest every element of the claimed invention. Further, in many instances, the Patent Office has not even attempted to provide specific support from the cited references for many novel elements of the claimed invention.

The following discusses in more detail the deficiencies of *Baggiolini* and *Abdaimi* regarding the present claims.

2. There is no suggestion or motivation to combine the cited references to arrive at Appellant's claimed invention

Appellant respectfully submits that the Patent Office has failed to establish a *prima facie* case of obviousness with respect to the rejection of Claims 1, 3-5, 8-12, 23-26, 28-37 and 39-40 under 35 U.S.C. § 103. Further, the Patent Office cannot simply use Appellant's disclosure as a guide for "building" Appellant's invention from various pieces of disparate art. There must be teaching, suggestion or motivation in the art for the combination of the cited references, and it is respectfully submitted that the Patent Office has failed to provide sufficient teaching, suggestion or motivation in its reliance on the cited art in maintaining its §103 rejection. Further, the examiner has not proven a reasonable expectation of success for the claimed method from the cited references.

The present invention provides, in part, a method of treating SCC 2/88, a canine squamous carcinoma cell line, for cancer, comprising the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic agent comprising a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof. The present invention also provides, in part, a method of administering a therapeutic or pharmaceutical agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof, said method comprising providing a pet food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet.

The Appellant has conducted research to show that vitamin D analogs inhibit proliferation and promote differentiation in canine cancer cells. Ordinarily, parenteral administration of vitamin D analogs to pets would necessitate the involvement of a veterinarian, which would substantially increase the expense. The Appellant has found, however, that enteral administration of vitamin D analogs is also effective for cancer therapy in dogs, and that incorporating vitamin D analogs into dog food is an effective and practical way of routinely administering vitamin D analogs to a pet suffering illnesses such as cancer.

Appellant does not believe that one skilled in the art would be inclined to modify and/or combine *Baggiolini* in view of *Abdaimi* to arrive at the claimed invention. For example,

Baggiolini teaches treating hyper-proliferative skin diseases such as psoriasis and basal cell carcinoma in a warm-blooded animal, i.e., a human, comprising administering an effective amount of certain vitamin D analogs. See, *Baggiolini*, column 11, lines 15-65. Further, *Baggiolini* only discloses specific vitamin D analogs for certain treatments such as skin diseases and neoplastic diseases. See, *Baggiolini*, columns 3-4. The Patent Office cannot use *Baggiolini* as a prior art reference to cover all vitamin D analogs, especially considering the wide variability of vitamin D analogs and the undiscovered and unique health attributes each vitamin D analog may have. Indeed, *Baggiolini* fails to disclose or even suggest the specific vitamin D analogs of Appellant's claimed invention. *Baggiolini* also fails to disclose or suggest the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic or pharmaceutical agent comprising a vitamin D analog as required by the present claims. Accordingly, *Baggiolini* is deficient with respect to the claimed invention.

Abdaimi fails to remedy the deficiencies of *Baggiolini* with respect to Appellant's claimed invention. *Abdaimi* teaches the use of EB1089 (1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3) as an antiproliferative and prodifferentiative agent. Specifically, *Abdaimi* describes a study that was conducted on nude mice implanted with a human epithelial cancer previously shown to produce high levels of PTHRP *in vitro*. See, *Abdaimi*, Abstract. The strategy employed was to use a continuous fusion of EB1089, which does not produce calcium elevation in control non-tumor-bearing animals. In accordance with this strategy, a pump was implanted adjacent the tumor to deliver a high concentration of the analogue to the tumor site. Nevertheless, *Abdaimi* fails to disclose or suggest the step of feeding a dog a dog food product comprising a proteinaceous component, a farinaceous component, and a therapeutic or pharmaceutical agent comprising a vitamin D analog as required by the present claims. In fact, *Abdaimi* clearly dictates that a continuous dosage be provided to the specific site so that elevated levels of calcium are not produced. See, *Abdaimi*, page 3328, first paragraph. This disclosure by *Abdaimi* actually teaches away from the application of feeding a dog a dog food containing an amount of a vitamin D analog according to the present claims because it would be impractical and undesirable to continuously feed a dog. Thus, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959).

References must be considered as a whole and those portions teaching against or away from the proposed combination must be considered. *Bausch & Lomb, Inc. v. Barnes-Hind/Hydrocurve Inc.*, 796 F.2d 443 (Fed. Cir. 1986). "A prior art reference may be considered to teach away when a person of ordinary skill, upon reading the reference would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the Applicant." *Monarch Knitting Machinery Corp. v. Fukuhara Industrial Trading Co., Ltd.*, 139 F.3d 1009 (Fed. Cir. 1998), quoting, *In re Gurley*, 27 F.3d 551 (Fed. Cir. 1994). Consequently, there is no teaching or suggestion within *Baggiolini* and *Abdaimi* or within the general knowledge of those skilled in the art that would have led one skilled in the art to make Appellant's claimed invention.

Furthermore, Appellant respectfully submits that the claimed method involves more than "routine practice for providing such medication to animals" as alleged in the Office Action at page 4. Appellant respectfully submits that the Patent Office has failed to even provide any specific reference indicating a reasonable expectation of success from feeding a dog a dog food containing the compounds recited in independent Claims 1, 26 and 34.

To support the combination and/or modification of the cited art to arrive at the claimed invention, the Patent Office has applied hindsight reasoning by selectively piecing together teachings of each of the references in an attempt to recreate what the claimed invention discloses. Indeed, Appellant respectfully submits that it is only with a hindsight reconstruction of Appellant's claimed invention that the Patent Office is able to even attempt to piece together a rejection of the claims. Of course, the Court of Appeals for the Federal Circuit has criticized this motivation to combine analysis (e.g., hindsight reconstruction) because the motivation to combine the references was first disclosed in the present invention. *In re O'Farrell,* 853 F.2d, 894, 902-903 (Fed. Cir. 1988).

3. *Baggiolini* and *Abdaimi*, Alone or in Combination, Fail to Disclose or Suggest Certain Claimed Features

The cited references, alone or in combination, fail to disclose or suggest a number of elements of the present claims. Independent Claim 1 provides, in part, a method of treating SCC 2/88, a canine squamous carcinoma cell line, for cancer, comprising the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic

agent comprising a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof. Independent Claims 26 and 34 provide, in part, a method of administering a therapeutic or pharmaceutical agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof, said method comprising providing a pet food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet.

In contrast to Appellant's presently claimed invention, *Baggiolini* fails to disclose or even suggest the specific vitamin D analogs of Appellant's claimed invention. Instead, *Baggiolini* discloses different vitamin D analogs for certain treatments such as skin diseases and neoplastic diseases. See, *Baggiolini*, columns 3-4. As discussed previously, the Patent Office cannot use *Baggiolini* as a prior art reference to cover all vitamin D analogs, especially considering the wide variability of vitamin D analogs and the undiscovered and unique health attributes each vitamin D analog may have. Further, *Baggiolini* also fails to disclose or even suggest the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic agent comprising a vitamin D analog as required by Appellant's claimed invention.

Abdaimi also fails to disclose or suggest the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic agent comprising a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof as disclosed in independent Claim 1. *Abdaimi* fails to disclose or suggest a method of administering a therapeutic or pharmaceutical agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof, said method comprising providing a pet food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet as disclosed, in part, in Claims 26 and 34. Accordingly, even if combinable, all of the claimed elements are not taught or suggested by *Baggiolini* or *Abdaimi*. For these reasons, the obviousness rejections of independent Claims 1, 26 and 34 are improper.

Further, Claims 6 and 28 are directed, in part, to administering the vitamin D analog in combination with a bone agent, a cytotoxic agent, an immuno response regulating agent, an anti-inflammatory agent or combinations thereof. Dependent Claims 8-11, 29-32 and 39 are directed, in part, to the step of feeding the dog particular dosages of the vitamin D analog per day. Dependent Claim 18 is directed, in part, to administering the Vitamin D analog in combination with a bone agent comprising at least one of conjugated estrogens, conjugated estrogen equivalents, anti-estrogens, calcitonin, bisphosphonates, calcium supplements, calcium receptor agonists, cobalamin, pertusssis toxin, boron, dehydroepiandrosterone, activin and bone morphogenic protein. Dependent Claim 19 is directed, in part, to administering the Vitamin D analog in combination with a cytotoxic agent comprising at least one of estramustine phosphate, prednimustine, cisplatin, 5-fluoro-uracil, melphalan, hydroxyurea, mitomycin, idarubicin, methotrexate, adriamycin, daunomycin, cyclophosphamide, doxorubicin, vincristine and pregnisone. Dependent Claims 20-22 are directed, in part, to administering the Vitamin D analog in combination with an anti-inflammatory agent comprising at least one of a steroidal anti-inflammatory agent and a non-steroidal anti-inflammatory agent. Dependent Claims 23-25 and 35-37 are directed, in part, to the step of feeding the dog a therapeutic agent comprising a vitamin D analog comprises producing a pharmaceutical agent from admixture which includes at least one of a pharmaceutically acceptable organic carrier substance and a pharmaceutically acceptable inorganic carrier substance.

Baggiolini and *Abdaimi*, alone or in combination, fail to teach or suggest any of these recited elements covered by the dependent claims. Further, the Patent Office has not even attempted to provide specific support from the cited references for these novel elements of the claimed invention. In fact, the Patent Office admits the cited references fail to disclose or suggest these elements. See, Office Action, pages 3-4. Instead, the Patent Office takes the easy route of improperly concluding that these recited elements are all obvious without providing further support from any particular source. However, a statement that modifications of the prior art to meet the claimed invention would have been "well within the ordinary skill of the art at the time the claimed invention was made" is not sufficient by itself to establish *prima facie* obviousness. See, MPEP 2143.01.

For the foregoing reasons, Appellant submits that the cited references do not disclose, teach or suggest independent Claims 1, 26 and 34, and the rejection of these claims should be reversed accordingly.

With respect to dependent Claims 3-5, 8-12, 23-25, 28-33, 35-37 and 39-40, the Appellant submits that these claims are allowable on their merits and also for the reasons presented above with respect to independent Claims 3-5, 8-12, 23-25, 28-33, 35-37 and 39-40.

C. THE REJECTION OF CLAIMS 6 AND 18-22 UNDER 35 U.S.C. §103(a) TO *BAGGIOLINI, ABDAIMI, KATZUNG* AND *HARDMAN* SHOULD BE REVERSED BECAUSE THE PATENT OFFICE HAS NOT ESTABLISHED A *PRIMA FACIE* CASE OF OBVIOUSNESS

Appellant respectfully submits that the obviousness rejection of Claims 6 and 18-22 should be reversed because the Patent Office fails to establish a *prima facie* case of obviousness. Regarding Claims 6 and 18-22, the Patent Office in the Final Office Action at page 5 alleges that the combination of *Baggiolini*, *Abdaimi*, *Katzung* and *Hardman* renders obvious the claimed invention. However, the Patent Office fails to establish a *prima facie* case of obviousness in each rejection because the primary references (*Baggiolini* and *Abdaimi*) are deficient with respect to the claimed invention, and *Katzung* and *Hardman* fail to remedy the deficiencies of the claimed invention.

As discussed previously, *Baggiolini* and *Abdaimi*, alone or in combination, fail to disclose or suggest the elements of Appellant's claimed invention with respect to independent Claims 1, 26 and 34. *Katzung* and *Hardman* fail to remedy the deficiencies of *Baggiolini* and *Abdaimi* with respect to Claims 1, 26 and 34. For example, *Katzung* and *Hardman* fail to disclose or suggest the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic agent comprising a vitamin D analog selected from the group consisting of $1\alpha,25\text{-}(OH)_2D_3$, $1\alpha,25\text{-}(OH)_2\text{-}16\text{-ene-}23\text{-yne-}D_3$ and $1\alpha,25\text{-}(OH)_2\text{-}22,24\text{-diene-}24,26,27\text{-trihomo-}D_3$ and stereoisomers thereof as disclosed in independent Claim 1. *Katzung* and *Hardman* also fail to disclose or suggest a method of administering a therapeutic or pharmaceutical agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of $1\alpha,25\text{-}(OH)_2D_3$, $1\alpha,25\text{-}(OH)_2\text{-}16\text{-ene-}23\text{-yne-}D_3$ and $1\alpha,25\text{-}(OH)_2\text{-}22,24\text{-diene-}24,26,27\text{-trihomo-}D_3$ and stereoisomers thereof, said method comprising providing a pet

food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet as disclosed, in part, in Claims 26 and 34. In fact, *Katzung* and *Hardman* have nothing to do nothing to do with vitamin D analogs or their uses in a pet food as recited in the present claims.

Further, the cited references, alone or in combination, fail to disclose or suggest the Claims 6 and 18-22 in conjunction with independent Claim 1 from which Claims 6 and 18-22 depend from. Claim 6 is directed, in part, to administering the vitamin D analog in combination with a bone agent, a cytotoxic agent, an immuno response regulating agent, an anti-inflammatory agent or combinations thereof. Dependent Claim 18, in part, is directed to administering the Vitamin D analog in combination with a bone agent comprising at least one of conjugated estrogens, conjugated estrogen equivalents, anti-estrogens, calcitonin, bisphosphonates, calcium supplements, calcium receptor agonists, cobalamin, pertusssis toxin, boron, dehydroepiandrosterone, activin and bone morphogenic protein. Dependent Claim 19 is directed, in part, to administering the Vitamin D analog in combination with a cytotoxic agent comprising at least one of estramustine phosphate, prednimustine, cisplatin, 5-fluoro-uracil, melphalan, hydroxyurea, mitomycin, idarubicin, methotrexate, adriamycin, daunomycin, cyclophosphamide, doxorubicin, vincristine and pregnisone. Dependent Claims 20-22 are directed, in part, to administering the Vitamin D analog in combination with an anti-inflammatory agent comprising at least one of a steroidal anti-inflammatory agent and a non-steroidal anti-inflammatory agent.

As discussed previously, *Baggiolini* and *Abdaimi*, alone or in combination, fail to disclose or suggest the elements of dependent Claims 6 and 18-22. The Patent Office even admits same. See, Office Action, page 5. *Katzung* and *Hardman* also fail to disclose or suggest these elements. The Patent Office uses *Katzung* for a rote listing of ingredients without showing any connection to a vitamin D analog for the treatment of cancer in a pet or addition to a pet food. Further, the Patent Office also fails to show the motivation to be combine *Katzung* with *Baggiolini* and *Abdaimi*.

Katzung generally teaches about hypercalcemia. Nonetheless, *Katzung* is a non-analogous reference because it does not relate to a method of treating SCC 2/88, a canine squamous carcinoma cell line. *Hardman* is only a page from a textbook indicating that pain is associated with cancer. Consequently, *Katzung* and *Hardman* fail to disclose or suggest any of

the compounds in Claims 6 and 18-22 in combination with a vitamin D analog as required by Claims 6 and 18-22.

For the foregoing reasons, the Appellant submits that dependent Claims 6 and 18-22 are allowable on their merits and also for the reasons presented above with respect to independent Claim 1. Further, Appellant respectfully submits that the patentability of Claim 1 renders moot the obviousness rejection of Claims 6 and 18-22. In this regard, the cited art fails to teach or suggest the elements of Claims 6 and 18-22 in combination with the novel elements of Claim 1.

IX. Conclusion

Appellant's claimed invention set forth in Claims 1, 3-6, 8-12, 18-26, 28-37 and 39-40 is neither disclosed, taught nor suggested by the cited references, either alone or in combination. The Patent Office has failed to establish a *prima facie* case of obviousness with respect to the rejection of the claimed invention. Accordingly, Appellant respectfully submits that the obviousness rejection is erroneous in law and in fact and should therefore be reversed by this Board.

Respectfully submitted,

BELL, BOYD & LLOYD LLC

BY _____

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Dated: April 13, 2005

CLAIMS APPENDIX

PENDING CLAIMS ON APPEAL OF U.S. PATENT APPLICATION SERIAL NO. 09/804,111

Claim 1 (previously presented): A method of treating SCC 2/88, a canine squamous carcinoma cell line, for cancer, comprising the step of feeding a dog a dog food comprising a proteinaceous component, a farinaceous component, and a therapeutic agent comprising a vitamin D analog selected from the group consisting of $1\alpha,25\text{-}(OH)_2D_3$, $1\alpha,25\text{-}(OH)_2\text{-}16\text{-ene-}23\text{-yne-}D_3$ and $1\alpha,25\text{-}(OH)_2\text{-}22,24\text{-diene-}24,26,27\text{-trihomo-}D_3$ and stereoisomers thereof.

Claim 3 (previously presented): The method of claim 1, wherein the vitamin D analog is $1\alpha,25\text{-}(OH)_2D_3$ and stereoisomers thereof.

Claim 4 (previously presented): The method of claim 1, wherein the vitamin D analog is $1\alpha,25\text{-}(OH)_2\text{-}16\text{-ene-}23\text{-yne-}D_3$ and stereoisomers thereof.

Claim 5 (previously presented): The method of claim 1, wherein the vitamin D analog is $1\alpha,25\text{-}(OH)_2\text{-}22,24\text{-diene-}24,26,27\text{-trihomo-}D_3$ and stereoisomers thereof.

Claim 6 (original): The method of claim 1, wherein the vitamin D analog is administered in combination with a bone agent, a cytotoxic agent, an immuno response regulating agent, an antiinflammatory agent or combinations thereof.

Claim 8 (original): The method of claim 1, wherein the dog is fed from about 0.025 to about 500 nmol/kg of body weight of the patient per day of the vitamin D analog.

Claim 9 (original): The method of claim 1, wherein the dog is fed from about 0.025 to about 100 nmol/kg of body weight of the patient per day of the vitamin D analog.

Claim 10 (original): The method of claim 1, wherein the dog is fed from about 0.025 to about 10 nmol/kg of body weight of the patient per day of the vitamin D analog.

Claim 11 (original): The method of claim 1, wherein the dog is fed from about 0.025 to about 1.0 nmol/kg of body weight of the patient per day of the vitamin D analog.

Claim 12 (original): The method of claim 1, wherein the dog is fed a therapeutically efficacious dosage of a vitamin D analog.

Claim 18 (previously presented): The method of claim 1 wherein the Vitamin D analog is administered in combination with a bone agent comprising at least one of conjugated estrogens, conjugated estrogen equivalents, anti-estrogens, calcitonin, bisphosphonates, calcium supplements, calcium receptor agonists, cobalamin, pertusssis toxin, boron, dehydroepiandrosterone, activin and bone morphogenic protein.

Claim 19 (previously presented): The method of claim 1 wherein the Vitamin D analog is administered in combination with a cytotoxic agent comprising at least one of estramustine phosphate, prednimustine, cisplatin, 5-fluoro-uracil, melphalan, hydroxyurea, mitomycin, idarubicin, methotrexate, adriamycin, daunomycin, cyclophosphamide, doxorubicin, vincristine and pregnisone.

Claim 20 (previously presented): The method of claim 1 wherein the Vitamin D analog is administered in combination with an anti-inflammatory agent comprising at least one of a steroidal anti-inflammatory agent and a non-steroidal anti-inflammatory agent.

Claim 21 (previously presented): The method of claim 20 wherein the steroidal anti-inflammatory agent includes corticosteroids.

Claim 22 (previously presented): The method of claim 20 wherein the non-steroidal anti-inflammatory agent includes at least one of salicylates and naproxen.

Claim 23 (previously presented): The method of claim 1 wherein feeding the dog a therapeutic agent comprising a vitamin D analog comprises producing a pharmaceutical agent

from admixture which includes at least one of a pharmaceutically acceptable organic carrier substance and a pharmaceutically acceptable inorganic carrier substance.

Claim 24 (previously presented): The method of claim 23 wherein the pharmaceutically acceptable organic carrier substance and the pharmaceutically acceptable inorganic carrier substance include at least one of water, salt and buffer solutions, alcohols, gum arabic, mineral and vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylase or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxyl methylcellulose, and polyvinyl pyrrolidine.

Claim 25 (previously presented): The method of claim 23 further comprising mixing the pharmaceutical agent with an auxiliary agent, the auxiliary agent including one or more of lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic active compounds.

Claim 26 (previously presented): A method of providing a therapeutic agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof, said method comprising providing a pet food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet.

Claim 28 (previously presented): The method of claim 26 wherein the vitamin D analog is administered in combination with at least one of a bone agent, a cytotoxic agent, and immuno response regulating agent, and an anti-inflammatory agent.

Claim 29 (original): The method of Claim 26, wherein the dog is fed from about 0.025 to about 500 nmol/kg of body weight of the dog per day of the vitamin D analog.

Claim 30 (original): The method of Claim 26, wherein the dog is fed from about 0.025 to about 100 nmol/kg of body weight of the dog per day of the vitamin D analog.

Claim 31 (original): The method of Claim 26, wherein the dog is fed from about 0.025 to about 10 nmol/kg of body weight of the dog per day of the vitamin D analog.

Claim 32 (original): The method of Claim 26, wherein the dog is fed from about 0.025 to about 1.0 nmol/kg of body weight of the dog per day of the vitamin D analog.

Claim 33 (original): The method of Claim 26, wherein the dog is fed a therapeutically efficacious dosage of the vitamin D analog.

Claim 34 (previously presented): A method of administering a pharmaceutical agent to a pet, wherein the agent comprises a vitamin D analog selected from the group consisting of 1α,25-$(OH)_2D_3$, 1α,25-$(OH)_2$-16-ene-23-yne-D_3 and 1α,25-$(OH)_2$-22,24-diene-24,26,27-trihomo-D_3 and stereoisomers thereof, said method comprising providing a pet food including a proteinaceous component, a farinaceous component, and the agent, and feeding the pet food to a pet.

Claim 35 (previously presented): The method of Claim 34 wherein feeding the pet food to a pet comprises producing a pharmaceutical agent from an admixture which includes at least one of a pharmaceutically acceptable organic carrier substance and a pharmaceutically acceptable inorganic carrier substance.

Claim 36 (previously presented): The method of Claim 35 wherein the pharmaceutically acceptable organic carrier substance and the pharmaceutically acceptable inorganic carrier substance include at least one of water, salt (buffer) solutions, alcohols, gum arabic, mineral and vegetable oils, benzyl alcohols, polyethylene glycols, gelatine, carbohydrates such as lactose, amylase or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, hydroxyl methyl cellulose, and polyvinyl pyrrolidone.

Claim 37 (previously presented): The method of Claim 35 further comprising mixing the pharmaceutical agent with an auxiliary agent that includes one or more of lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic active compounds.

Claim 39 (previously presented): The method of Claim 34 wherein the dog is fed from about 0.025 to about 500 nmol/kg of body weight of the dog per day of the vitamin D analog.

Claim 40 (previously presented): The method of Claim 34 wherein the dog is fed a therapeutically efficacious dosage of a vitamin D analog.

EVIDENCE APPENDIX

Office Action dated August 26, 2004 (Exhibit A)

U.S. Patent No. 5,087,619 to Baggiolini et al. ("*Baggiolini*"), cited by the Examiner in the Office Action dated August 26, 2004 (Exhibit B)

Cancer Research, 1999; 59: 3325-3328 by Abdaimi et al. ("*Abdaimi*"), cited by the Examiner in the Office Action dated August 26, 2004 (Exhibit C)

Basic and Clinical Pharmacology, 1995, p. 537-538, 661-663, 830-832, 838 and 841 by Katzung ("*Katzung*"), cited by the Examiner in the Office Action dated August 26, 2004 (Exhibit D)

Goodman and Gilman's The Pharmacological Basis of Therapeutics, 1996, p. 539 by Hardman et al. ("*Hardman*"), cited by the Examiner in the Office Action dated August 26, 2004 (Exhibit E)

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/804,111	03/12/2001	Nongnuch Inpanbutr	06204-00102	8670

7590 08/26/2004

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EXAMINER	
HUI, SAN MING R	

ART UNIT	PAPER NUMBER
1617	

DATE MAILED: 08/26/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/804,111	INPANBUTR, NONGNUCH	
	Examiner	Art Unit	
	San-ming Hui	1617	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 May 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6,8-12,18-26,28-37,39 and 40* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,8-12,18-26,28-37,39 and 40* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendments filed May 6, 2004 have been entered.

Claims 1-6, 8-12, 18-26, 28-37, and 39-40 are pending.

The outstanding objection is withdrawn in view of the amendments filed May 6, 2004.

The outstanding rejections under 35 USC 112, first and second paragraph are withdrawn in view of the amendments filed May 6, 2004.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-5, 8-12 and 23-26, 28-37, and 39-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boggolini et al. (USPN 5,087,619) and Abdaimi et al.

(Cancer Research, 1999; 59:3325-3328), insofar as it relates to the *in vivo* method of treating squamous carcinoma.

Boggiolini et al. teaches a method of treating neoplastic diseases in a warm-blooded animal comprising administering an effective amount of a vitamin D3 analogue (e.g., 1,25 (OH)2 D3 and 1,2-16 delta-23-yne-D3) (see for example tables III and IV, claim 20 and abstract). Boggiolini et al. also teaches the vitamin D compounds therein are useful in effectively against human squamous carcinoma cell lines (See col. 15, line 32-48, Table III). Boggiolini et al. also teaches vitamin D3 analogues in oral dosage forms such as capsules, see col. 21, lines 37-40. Pharmaceutically acceptable carrier materials may be incorporated in capsules, such as starch, magnesium stearate, lactose, peppermint oil (flavoring agent) (see in particular col. 21, line 37 to col. 22, line 27). Boggiolini et al. also teaches that the dosage for the vitamin D3 analogues is 0.1 to 10 microgram per day (see col. 11, lines 16-24).

Abdaimi et al. teaches that EB1089 is known to inhibit cell proliferation and is useful against squamous carcinoma (See page 3327, col. 2, third paragraph).

Boggolini et al. and Abdaimi et al. taken together do not teach the doses claimed herein in terms of nmol/Kg, neither do they teach all the pharmaceutical excipients and auxillaries claimed herein. Boggolini et al. and Abdaimi et al. also do not teach the method of treating SCC 2/88 cell lines. Boggolini et al. and Abdaimi et al. taken together do not teach the administration of the active through feed the dog with pet food with the herein claimed materials.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ/express the amounts of active in terms of nmol/Kg. It would have also been obvious to employ any known pharmaceutical excipients and auxillaries in the composition employed in the instant method. It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ the herein recited vitamin D compounds to treat squamous cell carcinoma. It would have been obvious to one of ordinary skill in the art at the time the invention was made to administer the active through feeding the active to the animal along with food substance.

One of ordinary skill in the art would have been motivated to employ/express the amounts of active in terms of nmol/Kg because optimization of amounts is within the skill of the artisan and is therefore obvious. Similarly the employment of any known pharmaceutical excipient and/or auxillaries with a known active is within the skill of the artisan and therefore obvious. Furthermore, providing the pet with medication through feed is considered a routine practice for providing medication to animals. Therefore, absent any evidence to the contrary, such method step is rendered obvious by the cited prior arts.

One of ordinary skill in the art would have been motivated to employ the herein recited vitamin D compounds to treat squamous cell carcinoma. The preferred vitamin D compounds herein are known to be useful in treating human squamous carcinoma. Employing the same compounds for treating a canine squamous carcinoma would be reasonably expected to be successful since the compounds herein are known to be

useful in any warm-blooded animal for fighting against squamous carcinoma, absent evidence to the contrary (See Boggolini et al.).

Claims 6 and 18-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boggolini et al. and Abdaimi et al. as applied to claims 1-5 and 7-12 and in further view of Katzung (Basic and Clinical Pharmacology, p.661-663, 838, 841, 830-832 and 537-538) and Hardman et al. (Goodman and Gilman's The Pharmacological Basis of Therapeutics, p.539), all of record in the previous office action.

Boggiolini et al. and Abdaimi et al. suggest the method of treating canine squamous carcinoma by employing the herein claimed vitamin D compounds.

Boggiolini et al. and Abdaimi et al. taken together do not teach the inclusion of a second active (i.e., bone agent, cytotoxic agent or anti inflammatory agent) in a composition employed in a method of treating cancer. Boggolini et al. and Abdaimi et al. taken together do not teach the administration of the active through feed the dog with pet food with the herein claimed materials.

Katzung teaches that hypercalcemia is a consequence of hypervitaminosis D. Katzung further teaches that bisphosphonates, calcitonin are employed in treating hypercalcemia, see pages 661-663. Katzung also teaches the employment of estrogen inhibitors, mitomycin, vincristine, doxorubicin, fluorouracil for treating different cancers, see page 838 and 841. Katzung further teaches cisplatin, melphalan, and methoxorate as anti-cancer agents, see pages 830-832. Both Salicylates and Naproxen are known NSAIDS (known for their anti-inflammatory and analgesic properties), 537-538.

Hardman et al. teaches that pain is commonly associated with cancer, see page 539.

It would have been obvious to one of ordinary skill at the time the invention at the time the invention was made to employ a second active (i.e., bone agent, cytotoxic agent or anti inflammatory agent) in a composition employed in a method of treating cancer. It would have been obvious to one of ordinary skill in the art at the time the invention was made to administer the active through feeding the active to the animal along with food substance.

One of ordinary skill in the art would have been motivated to employ bisphosphonates and calcitonin in a method of treating cancer employing a vitamin D3 analogue/derivative because they are known to be employed in methods of preventing and/or treating hypercalcemia associated with vitamin D administration. One of ordinary skill in the art would have been motivated to employ Salicylates and Naproxen, known NSAIDS, known for their anti-inflammatory and analgesic properties, in a method of treating cancer because pain is known to be associated with cancer. Furthermore, providing the pet with medication through feed is considered a routine practice for providing medication to animals. Therefore, absent any evidence to the contrary, such method step is rendered obvious by the cited prior arts.

One of ordinary skill in the art would have been motivated to employ estrogen inhibitors, mitomycin, vincristine, doxorubicin, fluorouracil cisplatin, melphalan, and methoxorate along with Vitamin D derivatives in a method of treating cancer. Estrogen inhibitors, mitomycin, vincristine, doxorubicin, fluorouracil cisplatin, melphalan, and

methoxorate are known to be employed in methods of treating cancer. Combining two agents which are known to be useful to treat cancer individually into a single composition useful for the very same purpose (i.e. treating cancer) is prima facie obvious (See *In re Kerkhoven* 205 USPQ 1069).

Response to Arguments

Applicant's rebuttal arguments in pages 7-9 averring the cited prior art's failure to teach the method step of providing the pet food with medication have been considered, but are not found persuasive. Providing the pet with medication through feed is considered a routine practice for providing medication to animals. Therefore, absent any evidence to the contrary, such method step is rendered obvious by the cited prior arts.

Applicant's rebuttal arguments averring the cited prior art's failure to provide motivation to combine the teachings of the prior art have been considered. The motivation to combine is based on the fact that the herein claimed agents are useful in treating cancer. It flows logically to concomitantly employ both agents, which are known to be useful for treating cancer, for the method of treating the very same disease, absent evidence to the contrary.

Applicant's arguments filed May 6, 2004 averring the individual cited references not teaching the instant invention have been fully considered but they are not persuasive. In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413,

208 USPQ 871 (CCPA 1981); *In re Merck & Co.*, 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). In the instant case, when taken the cited prior art together as a whole, one of ordinary skill in the art would have been reasonably expected to employ the herein claimed compounds to a method of treating a squamous cell carcinoma cell line.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to San-ming Hui whose telephone number is (571) 272-0626. The examiner can normally be reached on Mon 9:00 to 1:00, Tu - Fri from 9:00 to 6:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sreeni Padmanabhan, PhD., can be reached on (571) 272-0629. The fax

phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

San-ming Hui
Patent Examiner
Art Unit 1617

[CANCER RESEARCH 59, 3325–3328, July 15, 1999]

Advances in Brief

Reversal of Hypercalcemia with the Vitamin D Analogue EB1089 in a Human Model of Squamous Cancer[1]

Khadija El Abdaimi, Vasiliou Papavasiliou, Shafaat A. Rabbani, Johng S. Rhim, David Goltzman, and Richard Kremer[2]

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Abstract

EB1089, an analogue of 1,25 dihydroxyvitamin D with low calcemic activity is a potent inhibitor of parathyroid hormone-related peptide (PTHRP) production *in vitro*. The purpose of the present study was to determine whether EB1089 could reverse established hypercalcemia in BALB C nude mice implanted s.c. with a human epithelial cancer previously shown to produce high levels of PTHRP *in vitro*. Total plasma calcium was monitored before and after tumor development and increased steadily when the tumor reached ≥0.5 cm^3. When total calcium was ≥2.85 mmol/liter, animals were treated with a constant infusion of EB1089 or vehicle alone for a period of 2 weeks. A significant and sustained reduction of plasma calcium from 3.2 ± 0.1 to 2.7 ± 0.08 ($P < 0.01$) mmol/liter was observed during infusion with EB1089. In contrast, calcium levels in vehicle-treated animals continued to rise during the infusion period. Tumor growth velocity also slowed significantly after the administration of EB1089 as compared with vehicle-treated animals. Plasma PTHRP levels measured at the end of the 2 weeks' infusion period were significantly lower in animals treated with EB1089 as compared with animals treated with vehicle alone (44 ± 8 pg/ml *versus* 194 ± 35 pg/ml, $P < 0.001$). These results, therefore, demonstrate that EB1089 can reverse established hypercalcemia in a human model of squamous cancer.

Introduction

Previous studies (1, 2) have clearly demonstrated that $1,25(OH)_2D_3$[3] is a potent antiproliferative and prodifferentiative agent. These properties have been demonstrated *in vitro* not only in normal cells but also in cancer cells (3–5). *In vivo* studies (6) have also produced significant tumor regression in human tumors in nude mice. However, the therapeutic application of $1,25(OH)_2D_3$ is seriously limited by its side effects, which include hypercalcemia and hypercalciuria (7). Although its biological properties and its effect on tumor growth makes $1,25(OH)_2D_3$ a potential anticancer agent, its calcium-regulating properties would normally exclude it as a candidate for treating MAH, a condition frequently encountered in hospitalized patients (8). However, paradoxically we previously hypothesized that this obstacle may indeed be overcome by using vitamin D analogues with low calcemic activity. The rationale for using these analogues to treat MAH derives from our previous *in vitro* and *in vivo* studies as outlined below.

Hypercalcemia is associated with squamous cell cancers, which typically overproduce PTHRP (9). In normal cells, we previously demonstrated that $1,25(OH)_2D_3$ blocks PTHRP production (10). Using a multistep model of epithelial cell carcinogenesis, we demonstrated that the progression from the normal to the malignant phenotype was characterized by a partial resistance to the inhibitory effect by $1,25(OH)_2D_3$ requiring 10- to 100-fold higher concentrations of $1,25(OH)_2D_3$ to achieve the same effects (11, 12). To develop alternative strategies to block PTHRP production *in vitro* and *in vivo*, several $1,25(OH)_2D_3$ analogues—known to have low calcemic activities yet to retain strong antiproliferative effects on keratinocytes *in vitro* (13)—were tested. One such analogue, EB1089 (Leo Pharmaceuticals Ltd, Ballerup, Denmark), has a half-life similar to that of $1,25(OH)_2D_3$ yet is 10 times less potent in promoting hypercalcemia in rats (14). In the tumor progression model, EB1089 was 100 times more potent than $1,25(OH)_2D_3$ in inhibiting PTHRP (14). EB1089 is, therefore, not only a potential inhibitor of PTHRP overproduction *in vivo* but represents a possible new strategy in hypercalcemia therapy.

Subsequently, an animal model of MAH, the rat Leydig cell tumor H500 (15) was used. The hypercalcemic state associated with this rat testicular cancer has been linked to PTHRP (16, 17). Animals that were implanted with the rat Leydig cell tumor H500 and were treated simultaneously with a constant infusion of EB 1089 maintained normocalcemia and had lower circulating PTHRP concentrations than animals treated with the vehicle alone (18). These results clearly indicated that vitamin D analogues with low calcemic activities can prevent the development of hypercalcemia in an established animal model when administered at the time of tumor implantation. However, for these analogues to be useful clinically, it remains to be determined that they can reverse established hypercalcemia and that they can be applied to human models of MAH. Our present study was designed to closely mimic the clinical situation encountered in patients with MAH. In this experimental design, a human model of squamous cancer-producing PTHRP was used, and animals were treated after the onset of hypercalcemia.

Our present data clearly indicate that EB1089 efficiently blocks PTHRP production and reverses established hypercalcemia in nude mice implanted with human squamous tumors that express high levels of PTHRP.

Materials and Methods

Cell Culture Conditions. The HPK1A cell line was established from normal human keratinocytes by stable transfection with human papillomavirus type 16 (19). Despite acquiring an indefinite life span in culture, these cells retain differentiation properties characteristic of normal keratinocytes (20) and are nontumorigenic when injected into nude mice. These immortalized cells were subsequently transformed into the malignant HPK1A-*ras* cell line after transfection with a plasmid carrying an activated H-*ras* oncogene (12, 21). In addition to forming colonies in soft agar, the malignant HPK1A-*ras* cells produce squamous cell carcinoma when transplanted into nude mice. HPK1A-*ras* cell line was seeded and grown in DMEM (Life Technologies, Inc.)

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[3] The abbreviations used are: $1,25(OH)_2D_3$, 1,25 dihydroxyvitamin D_3; PTHRP, parathyroid hormone-related peptide; MAH, malignancy-associated hypercalcemia; FBS, fetal bovine serum; iPTHRP, immunoreactive PTHRP.

Table 1 *Properties of 1,25(OH)$_2$D$_3$ and EB1089*

Compounds	Relative calcemic activity	*In vivo* half-life
1,25(OH)$_2$D$_3$	1	2.4 h[a]
EB1089	0.4[b]	2.8 h[a]

[a] Binderup *et al.* (14).
[b] Mathiasen *et al.* (22).

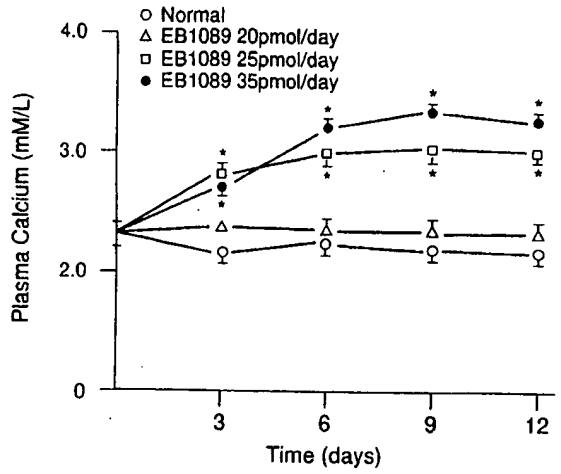

Fig. 1. Effect of EB1089 on plasma calcium in normal Balb C nude mice. Normal non-tumor-bearing animals were infused with 20, 25, or 35 pmol of EB1089 per 24 h or with vehicle alone (four animals per group) by constant infusion using Alzet osmotic minipumps. Blood was collected at timed intervals by orbital bleeding, and total plasma calcium was measured by microchemistry (Kodak Ektachem, Montreal, Quebec, Canada). Results represent the mean ± SE of three different experiments. *, significant difference ($P < 0.01$) in plasma calcium from control (vehicle alone) at each time point.

supplemented with 10% FBS (Life Technologies, Inc.) and maintained by serial passaging. Prior to s.c. implantation into nude mice, proliferating cells were trypsinized, washed in DMEM containing 10% FBS, and resuspended in complete medium.

For cell-growth experiments, cells were seeded at a density of 1×10^4 cells/well in 24-well cluster plates and grown to 20% confluence. After 24 h in basal conditions (DMEM without serum), fresh medium containing 10% FBS without or with varying concentrations of EB1089 was added to the cultured cells, and incubations were continued for 72 h. For survival assays, cells were treated with increasing concentrations of EB1089 without FBS. Cells were trypsinized and counted in a coulter counter (LKB, Montreal, Quebec, Canada). XTT-Microculture tetrazolium assay for cell growth was done as described previously (12). Cells were seeded at 2×10^3 cells/100 μl into 96-well microtiter plates using the same conditions as described above for cell growth. Formazan production was measured at 490 nm using a Bio-Rad microplate reader. Results were then expressed as percent of FBS-stimulated growth.

Vitamin D Analogue. EB1089 was kindly provided by Leo Pharmaceuticals (14). EB1089 has terminal ethyl groups and double binds (at positions 22 and 24) in the side chain. This compound has low calcemic activity (14) and a half-life similar to 1,25(OH)$_2$D$_3$ *in vivo* (Ref. 22; Table 1).

Animal Protocols. BalbC nude mice (20 g; female) were implanted s.c. with 10^7 ras-transformed keratinocytes (HPK1Aras) as described previously (21) in 200–300 μl of suspension of complete medium (DMEM and 10% FBS).

All of the animals were examined twice a week for the development of a palpable tumor at the site of injection or other s.c. sites. Three-dimensional tumor measurements were done using calipers. Tumor diameters long axis (L) and mean mid axis width (W) were measured to estimate the tumor volume using the following formula:

$$\frac{4}{3}\Pi \times \left(\frac{L}{2}\frac{W}{2}\right)$$

Growth curves were generated by plotting the mean tumor volume of mice treated with EB1089 against mice treated with vehicle alone.

Preliminary experiments determined the minimum effective dosage of EB1089 that does not result in hypercalcemia in non-tumor-bearing animals. When the tumor-bearing animals developed hypercalcemia (total calcium >2.85 mmol/liter), osmotic minipumps (model 2004, Alza Corporation, Palo Alto, CA) were implanted under general anesthesia s.c. on the back of the animals immediately adjacent to the tumor site. Each minipump contained EB1089 dissolved in 0.1 mg/ml in polyethylene glycol: 0.05 M Na_2HPO_4 (80:20) to deliver a continuous dose of the compound for up to 2 weeks at a delivery rate of 2.5 μl/h. One group of tumor-bearing animals received vehicle alone. Each group consisted of eight animals.

Plasma Calcium and PTHRP Measurements. Plasma samples were obtained by orbital bleeding at regular intervals (every 5–7 days), and 50–100 μl were used to measure total calcium and albumin by microchemistry (Kodak Ektachrome).

Animals were killed by cardiac puncture, and 300 μl of plasma were recovered for measurement of plasma calcium and iPTHRP. PTHRP was measured using an immunoradiometric assay as described previously (23). The assay recognizes the intact first 86 amino acids of the molecule and has a detection limit of 2 pg/ml.

Statistical Analysis. All of the results are expressed as the mean ± SE, and statistical comparisons are made on the basis of Student's t test or a one-way ANOVA, using a Bonferroni adjustment when appropriate (24).

Results

Effect of EB1089 on Plasma Calcium. In non-tumor-bearing animals the lowest dose of EB1089 (20 pmol/24 h) did not produce

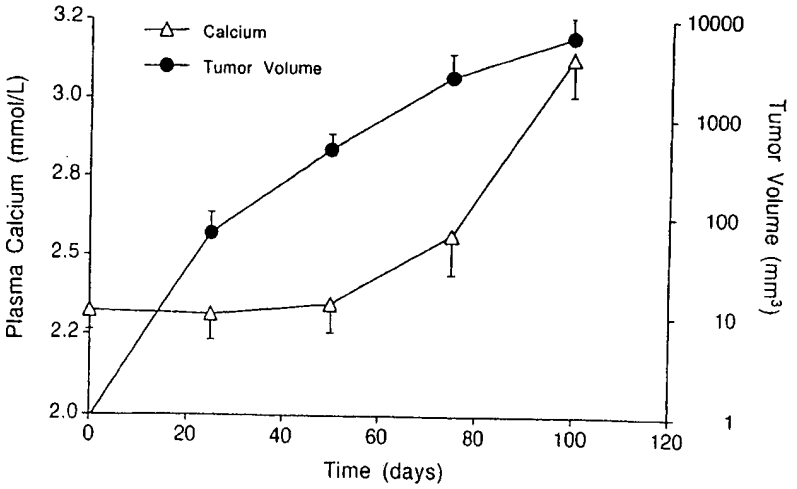

Fig. 2. Simultaneous analysis of tumor growth and plasma calcium. Balb C nude mice were implanted s.c. with 10^7 HPK1A-*ras* cells as described in "Materials and Methods." Tumor volume and plasma calcium were measured at timed intervals. Note that plasma calcium remains within the normal range until around 50 days after tumor implantation and starts to rise when the tumor size is above 500 mm^3.

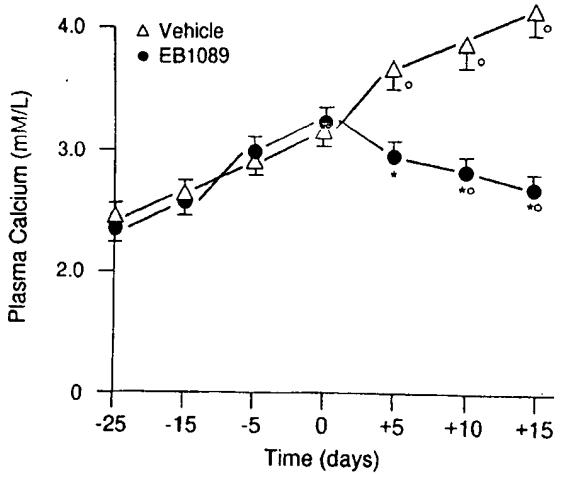

Fig. 3. Effect of EB1089 on plasma calcium in tumor-bearing animals. Balb C nude mice were infused at time 0 with 18 pmol of EB1089 per 24 h or with vehicle alone as described in Fig. 1. Blood was collected at timed intervals by orbital bleeding, and total plasma calcium was measured by microchemistry. Results represent the mean ± SE of eight animals in each group. Animals were treated at time 0 with vehicle alone (Δ-Δ) or with EB1089 (●-●). *, significant difference ($P < 0.01$) in plasma calcium from control tumor-bearing animals (vehicle alone) at the time points indicated (*+5, +15*). °, significant change ($P < 0.01$) from plasma calcium at time 0 (pretreatment).

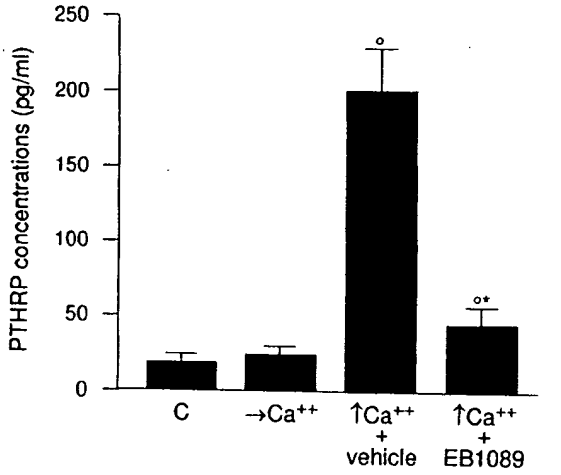

Fig. 4. Effect of EB1089 on plasma iPTHRP concentrations in tumor-bearing animals. Balb C nude mice were treated as described in Fig. 3. At the end of the infusion period (14 days), blood was collected by cardiac puncture, and plasma was kept at −70°C until assayed. PTHRP concentrations were determined using an immunoradiometric assay (DSL, Webster, Texas) specific for PTHRP 1–86. The detection limit of the assay is 2 pg/ml. Results represent the mean ± SE of eight animals in each group. *C*, controls, nontumor-bearing animals; $\rightarrow Ca^{++}$, normocalcemic tumor-bearing animals; $\uparrow Ca^{++}$, hypercalcemic tumor-bearing animals; *, significant difference ($P < 0.01$) from vehicle-treated animals; °, significant difference ($P < 0.01$) from C and $\uparrow Ca^{++}$.

hypercalcemia; however, higher doses produced a progressive increase in plasma calcium (Fig. 1). Consequently, hypercalcemic tumor-bearing animals were treated with a constant infusion of 18 pmol/24 h of EB1089.

After tumor cells implantation, a progressive increase in tumor volume was observed that preceded an increase in plasma calcium starting between 6 and 10 weeks after tumor implantation (Fig. 2). A significant increase in plasma calcium occurred when tumor volume was above 0.5 cm^3. When plasma calcium was ≥2.85 mmol/liter, animals were implanted with Alzet osmotic minipumps and received EB1089 (18 pmol/24 h) or vehicle alone. Vehicle-treated animals showed a continuous increase in their plasma calcium (Fig. 3). In contrast, tumor- bearing animals—infused with the analogue (18 pmol/24 h)—demonstrated a significant decrease in their plasma calcium. Furthermore, these animals reached plasma calcium levels comparable to the calcium levels of non-tumor-bearing mice (Fig. 3).

Effect on PTHRP Production. iPTHRP was measured in the plasma of non-tumor-bearing animals (control), untreated tumor-bearing animals, and hypercalcemic tumor-bearing animals treated with EB1089 or vehicle alone. iPTHRP was low and not significantly different between a group of eight non-tumor-bearing animals (controls) and a group of eight normocalcemic tumor-bearing animals (15 ± 4 pg/ml *versus* 18 ± 5 pg/ml; Fig. 4). Normocalcemic tumor-bearing animals with a tumor volume of <0.5 cm^3 were sacrificed 4–6 weeks after tumor implantation. All of the hypercalcemic tumor-bearing animals receiving vehicle alone and bled by cardiac puncture at the time of death had high iPTHRP plasma concentration (194 ± 35 pg/ml), whereas tumor-bearing animals receiving EB1089 and killed 2 weeks after the administration of the analogue had a significant reduction of PTHRP (44 ± 8 pg/ml, $P < 0.01$; Fig. 4).

Effect on Cell Proliferation *in Vitro* and Tumor Growth *in Vivo*. To further understand the mechanism of this effect, we performed *in vitro* studies for cell proliferation and survival assays (to assess apoptosis). These results are summarized in Table 2. In serum-treated cells, EB1089 significantly inhibited cellular growth in a dose-dependent fashion achieving a maximal inhibition at 10^{-7} M. However, no effect on cell survival was detected in serum-deprived experiments (data not shown).

Tumor growth velocity was assessed before and during infusion of EB1089 or vehicle alone (Table 2). Tumors continued to grow in both treated and control group. However, in EB1089-treated animals, tumor growth velocity decreased significantly as compared with control animals (16.8% ± 5% *versus* 95% ± 32%; $P < 0.05$).

Discussion

$1,25(OH)_2D_3$ analogues with low calcemic activities are of potential value as anticancer agents (13, 18, 25–28). These analogues retain strong antiproliferative effects, although less calcemic than $1,25(OH)_2D_3$. In previous studies, we have used one such analogue, EB1089, and demonstrated its strong capacity to inhibit PTHRP production *in vitro* (13) and *in vivo* (18). Our present data indicate that this analogue can also reverse established hypercalcemia in nude mice that have been implanted with a human squamous cancer. This tumor produces high levels of PTHRP (11, 13), a mediator linked to MAH of the majority of solid tumors in humans (9). Our previous demonstration (18) that EB1089 could be used as a preventative agent in the treatment of hypercalcemia in the rat Leydig cell tumor suggested that such an analogue could also be effective in reversing established hypercalcemia, a clinical situation frequently encountered in advanced cancer. Furthermore, a human model of epithelial carcinogenesis was chosen to closely mimic the human

Table 2 *Effect of EB1089 on FBS-stimulated cell growth in HPK1A-ras cells* in vitro *and on tumor growth* in vivo

After 24 h in basal conditions (no FBS), fresh medium containing FBS (10%) and either 10^{-7} or 10^{-9} M EB1089 was added to cultures of HPK1A-*ras* cells for 72 h. Cell growth was assessed after cell trypsinization using a coulter counter. Formazan production was assessed using the XTT assay (12). Each value represents the mean ± SD of six determinations and is representative of three separate experiments. Tumor volume was assessed before and after the administration of EB1089 or vehicle alone and as a percentage increase of pretreatment tumor volume.

	Cell proliferation		Tumor growth
EB1089 concentration (M)	Cell number % control	Formazan production	% increase of pretreatment size
Control (100%)			vehicle 95 ± 32
10^{-9}	86.4 ± 3.5[a]	92.3 ± 3.2[a]	EB1089 16.8 ± 5[a]
10^{-7}	82.4 ± 0.84[a]	78.3 ± 5.0[a]	

[a] Significant difference from control values or vehicle-treated animals ($P < 0.05$).

clinical syndrome of MAH. Our strategy was to use a continuous infusion of EB1089, which does not produce calcium elevation in control non-tumor-bearing animals. A dosage of 18 pmol/24 h was determined, and the pump was implanted adjacent to the tumor to deliver a high concentration of the analogue to the tumor site. This experimental design was favored to achieve maximal local inhibition of PTHRP production by tumor cells. Although we cannot exclude a strong systemic effect of EB1089, it is likely that this experimental design favors a strong local effect of EB1089 on the tumor. To be useful clinically, such agents will require adequate modes of delivery in cancer patients to mimic the experimental design presented here.

The administration of EB1089 was effective in reversing hypercalcemia in tumor-bearing animals in which calcium levels were ≥2.85 mmol/liter. This cutoff value was used because it represents the clinical situation in cancer patients in which hypercalcemia often requires treatment with antiresorptive agents such as bisphosphonates. Bisphosphonates are highly effective in reversing hypercalcemia but their effect are short-lived (29), and patients with elevated PTHRP levels are often resistant to these agents (30). Consequently, an agent that inhibits PTHRP production would represent a major advance in the treatment of this common condition. Our study clearly indicates the EB1089 blocks PTHRP production and decreases its levels to near normal values. Infusion of the analogue also significantly reduced the growth velocity of the tumor. However, tumor growth continued during treatment, and tumor volume was significantly higher after infusion than before EB1089 infusion indicating that plasma calcium reduction was not a direct result of tumor shrinkage. Our *in vitro* data indicate that the effect of EB1089 seen on tumor growth is secondary to a direct effect of the vitamin D analogue on cellular proliferation as previously reported for other cell types (26, 31). *In vitro* survival assays indicate that the effect of EB1089 is unlikely to be secondary to apoptotic cell death and correlates well with the absence of an *in vivo* effect on tumor shrinkage.

The mechanism(s) by which $1,25(OH)_2D_3$ and its analogues inhibit cell growth remains elusive. One postulated mechanism is that they modulate the expression of cell-cycle-associated genes. We and others have shown that $1,25(OH)_2D_3$ inhibit the expression of the c-*myc* oncogene (32–34) and also blocks the progression from G_0-G_1 to the S phase of the cell cycle (35, 36). Another potential mechanism is that EB1089 works independently via the inhibition of PTHRP. Indeed, PTHRP was previously reported to promote tumor growth both *in vitro* and *in vivo* (37), and we cannot exclude an indirect effect of EB1089 on tumor growth via a reduction of PTHRP production by tumor cells.

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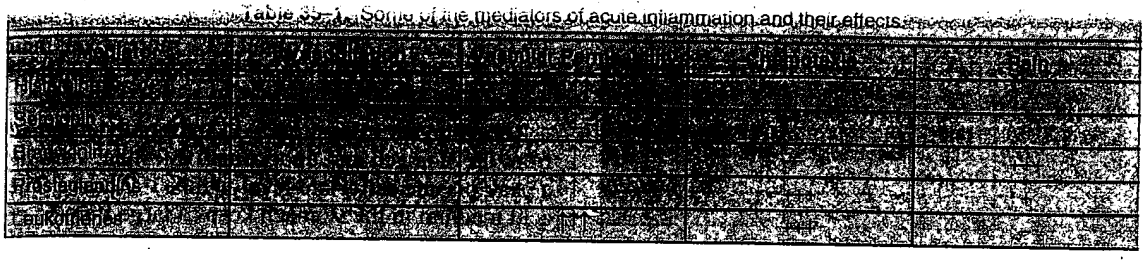
Table 35–1. Some of the mediators of acute inflammation and their effects.



of inflammatory arthritis. Unfortunately, the severe toxicity associated with chronic corticosteroid therapy prevents their use except in the control of acute flare-ups of joint disease. Therefore, the nonsteroidal anti-inflammatory drugs have assumed the major role in the treatment of arthritis.

Another important group of agents are characterized as slow-acting antirheumatic drugs (SAARDs) or disease-modifying antirheumatic drugs (DMARDs). Very little is known about their mechanisms of action. Unfortunately, they are considerably more toxic than the nonsteroidal anti-inflammatory agents.

I. NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Salicylates and other agents used to treat rheumatic disease share the capacity to suppress the signs and symptoms of inflammation. Some of the drugs also exert antipyretic and analgesic effects, but it is their anti-inflammatory properties that make them useful in the management of disorders in which pain is related to the intensity of the inflammatory process. Several of the newer NSAIDs are used for special indications. These include indomethacin, phenylbutazone, and ketorolac. They are discussed separately.

Table 35–2. Some of the mediators of chronic inflammation, eg, in rheumatoid arthritis.

Mediator	Sources	Primary Effects
Interleukins 1, 2, and 6	Macrophages, lymphocytes	Lymphocyte activation, prostaglandin production
GM-CSF[1]	T lymphocytes, endothelial cells, fibroblasts	Macrophage and granulocyte activation
TNF-α[2]	Macrophages	Prostaglandin production
Interferons	Macrophages, endothelial cells, T lymphocytes	Multiple
PDGF[3]	Macrophages, endothelial cells, fibroblasts, platelets	Fibroblast chemotaxis, proliferation

[1]Granulocyte-macrophage colony-stimulating factor.
[2]Tumor necrosis factor alpha.
[3]Platelet-derived growth factor.

ASPIRIN & OTHER SALICYLATES

Aspirin and all but one of the newer nonsteroidal anti-inflammatory drugs (NSAIDs) (ibuprofen, naproxen, etc) are related chemically in that they are weak organic acids; nabumetone is a ketone prodrug that is metabolized to an acidic active drug. They share the important property of inhibiting prostaglandin biosynthesis. They may also decrease the production of free radicals and of superoxide and may interact with adenylyl cyclase to alter the cellular concentration of cAMP. Although these drugs effectively inhibit inflammation, there is no evidence that—in contrast to drugs such as methotrexate and penicillamine—they alter the course of an arthritic disorder. Aspirin's long history of use and availability without prescription diminishes its glamour compared to that of the newer NSAIDs. However, because of its low cost and long history of safety, aspirin remains the initial drug of choice for treating the majority of articular and musculoskeletal disorders. Aspirin is also the standard against which all anti-inflammatory agents are measured (Table 35–3), and it should not be abandoned unless a specific contraindication exists or another NSAID offers a clearly demonstrable advantage.

History

Quinine from cinchona bark is one of the oldest remedies for relief of mild pain and fever. Willow bark was used in folk medicine for years for similar indications. In 1763, Reverend Edmund Stone, in a letter to the president of the Royal Society, described his success in treating fever with a powdered form of the bark of the willow. He had noted that the bitterness of willow bark was reminiscent of the taste of cinchona bark, the source of quinine. The active in-

Table 35–3. Properties of aspirin and some newer nonsteroidal anti-inflammatory drugs.

Drug	Half-life (hours)	Urinary Excretion of Unchanged Drug	Recommended Anti-inflammatory Dosage
Aspirin	0.25	<2%	1200–1600 mg tid
Salicylate	2–10	2–80%	See footnote 2
Apazone	15	62%	600 mg bid
Diclofenac	1.1	<1%	50–75 mg qid
Diflunisal	13	3–9%	500 mg bid
Elodolac	6.9	1%	200–300 mg qid
Fenoprofen	2.5	30%	600 mg qid
Flurbiprofen	3.8	1%	300 mg tid
Ibuprofen	2	1%	600 mg qid
Indomethacin	4–5	16%	50–70 mg tid
Ketoprofen	1.8	<1%	70 mg tid
Ketorolac	4–10	58%	10 mg qid[3]
Meclofenamate	1–3	2–4%	100 mg qid
Nabumetone[4]	26	1%	1000–2000 mg qd[5]
Naproxen	14	<1%	375 mg bid
Oxaprozin	58	1–4%	1200–1800 mg qd[5]
Phenylbutazone[6]	68	1–3%	100 mg qid
Piroxicam	57	4–10%	20 mg qd[5]
Sulindac	8	7%	200 mg bid
Tolmetin	1.1	7%	400 mg qid

[1]Major anti-inflammatory metabolite of aspirin.
[2]Salicylate is usually given in the form of aspirin.
[3]Recommended for treatment of acute (eg, surgical) pain only.
[4]Nabumetone is a prodrug; the half-life and urinary excretion are for its active metabolite
[5]A single daily dose is sufficient because of the long half-life.
[6]Phenylbutazone is not recommended for chronic use. The dose listed is for treatment of acute gouty arthritis and should not be given for more than one week. It may be preceded by a single loading dose of 400 mg.

gredient of willow bark, salicin, which on hydrolysis yields salicylic acid, was later found in other natural sources. Acetylsalicylic acid was synthesized in 1853, but the drug was not used until 1899, when it was found to be effective in arthritis and well tolerated. The name aspirin was coined from the German word for the compound, *a*cetyl*spir*säure (*Spirea,* the genus of plants from which it was obtained, and *Säure,* the German word for acid). Because of its greater efficacy and lower cost, aspirin rapidly replaced the natural products then in use and has remained one of the most widely employed remedies for over 90 years.

Chemistry & Pharmacokinetics

Salicylic acid is a simple organic acid with a pK_a of 3.0. Aspirin (acetylsalicylic acid; ASA) has a pK_a of 3.5 (see Table 1–2). It is about 50% more potent than sodium salicylate, although the latter compound causes less gastric irritation.

The salicylates (Figure 35–1) are rapidly absorbed from the stomach and upper small intestine, yielding a peak plasma salicylate level within 1–2 hours. The acid medium in the stomach keeps a large fraction of the salicylate in the nonionized form, promoting absorption. However, when high concentrations of salicylate enter the mucosal cell, the drug may damage the mucosal barrier. If the gastric pH is raised by a suitable buffer to 3.5 or higher, gastric irritation is minimized.

Aspirin is absorbed as such and is hydrolyzed to acetic acid and salicylate by esterases in tissue and blood. Salicylate is bound to albumin, but, as the serum concentration of salicylate increases, a greater fraction remains unbound and available to tissues. Ingested salicylate and that generated by the hydrolysis of aspirin may be excreted unchanged, but most is converted to water-soluble conjugates that are rapidly cleared by the kidney (Figure 35–1). When this pathway becomes saturated, a small increase in aspirin dose results in a large increase in plasma levels. Alkalinization of the urine increases the rate of excretion of free salicylate. When aspirin is used in low doses (600 mg), elimination is in accordance with first-order kinetics and the serum half-life is 3–5 hours. With higher dosage, zero-order kinetics prevail; at anti-inflammatory dosage (≥ 4 g/d), the half-life increases to 15 hours or more. This effect occurs in about a week and is related to saturation of hepatic enzymes that catalyze the formation of salicylate metabolites, salicylphenylglucuronide and salicyluric acid.

Pharmacodynamics

A. Mechanism of Action: The effectiveness of aspirin is largely due to its capacity to inhibit prostaglandin biosynthesis (see Chapter 18). It does this by irreversibly blocking the enzyme cyclooxygenase (prostaglandin synthase), which catalyzes the conversion of arachidonic acid to endoperoxide compounds; at appropriate doses, the drug decreases the formation of both the prostaglandins and thromboxane A_2 but not the leukotrienes (Figure 35–2). There is no evidence that aspirin is a selective inhibitor of COX II. Most of an anti-inflammatory dose of aspirin is rapidly deacetylated to form salicylate as the active metabolite. Salicylate reversibly inhibits prostaglandin synthesis.

B. Anti-inflammatory Effects: In addition to reducing the synthesis of eicosanoid mediators, aspirin also interferes with the chemical mediators of the kallikrein system (see Chapter 17). As a result, aspirin inhibits granulocyte adherence to damaged vasculature, stabilizes lysosomes, and inhibits the migration of polymorphonuclear leukocytes and macrophages into the site of inflammation.

mottling of the enamel proportionate to the concentration above 1 ppm.

Because of the general lack of effectiveness of other agents in stimulating new bone growth in patients with osteoporosis, interest in the use of fluoride for this disorder has been renewed (see Osteoporosis, page 666). Results of earlier studies indicated that fluoride alone without adequate calcium supplementation produced osteomalacia. More recent studies, in which calcium supplementation has been adequate, have demonstrated an improvement in calcium balance, an increase in bone mineral, and an increase in trabecular bone volume. However, two studies of the ability of fluoride to reduce fractures reached opposite conclusions (Riggs, 1990; Pak, 1989). Adverse effects observed—at the doses used for testing fluoride's effect on bone—include nausea and vomiting, gastrointestinal blood loss, arthralgias, and arthritis in a substantial proportion of patients. Such effects are usually responsive to reduction of the dose or giving fluoride with meals (or both). At present, fluoride is not approved by the Food and Drug Administration for use in osteoporosis.

Acute toxicity, generally due to ingestion of fluoride-containing rat poisons, includes gastrointestinal symptoms and neurologic signs of hypocalcemia presumably related to the calcium-binding properties of fluoride. Fluoride in acutely toxic doses may also cause cardiovascular collapse or respiratory failure. Chronic exposure to very high levels of fluoride dust in the inspired air results in **crippling fluorosis**, characterized by thickening of the cortex of long bones and bony exostoses, especially in the vertebrae.

II. CLINICAL PHARMACOLOGY

Disorders of bone mineral homeostasis generally present with abnormalities in serum or urine calcium levels (or both), often accompanied by abnormal serum phosphate levels. These abnormal mineral concentrations may themselves cause symptoms requiring immediate treatment (eg, coma in malignant hypercalcemia, tetany in hypocalcemia). More commonly, they serve as clues to an underlying disorder in hormonal regulators (eg, primary hyperparathyroidism), target tissue response (eg, chronic renal failure), or drug abuse (eg, vitamin D intoxication). In such cases, treatment of the underlying disorder is of prime importance.

Since bone and kidney play central roles in bone mineral homeostasis, conditions that alter bone mineral homeostasis usually affect either or both of these tissues secondarily. Effects on bone can result in osteoporosis (abnormal loss of bone; remaining bone histologically normal), osteomalacia (abnormal bone formation due to inadequate mineralization), or osteitis fibrosa (excessive bone resorption with fibrotic replacement of resorption cavities). Biochemical markers of skeletal involvement include changes in serum levels of the skeletal isoenzyme of alkaline phosphatase and osteocalcin (reflecting osteoblastic activity) and urine levels of hydroxyproline and pyridinoline cross-links (reflecting osteoclastic activity). The kidney becomes involved when the calcium-times-phosphate product in serum exceeds the point at which ectopic calcification occurs (often in renal parenchyma) or when the calcium-times-oxalate (or phosphate) product in urine exceeds saturation, leading to nephrocalcinosis and nephrolithiasis. Subtle early indicators of such renal involvement include polyuria, nocturia, and hyposthenuria. Radiologic evidence of nephrocalcinosis and stones is not generally observed until later. The degree of the ensuing renal failure is best followed by monitoring the decline in creatinine clearance.

ABNORMAL SERUM CALCIUM & PHOSPHATE LEVELS

HYPERCALCEMIA

Hypercalcemia causes central nervous system depression, including coma, and is potentially lethal. Its major causes (other than thiazide therapy) are hyperparathyroidism and cancer with or without bone metastases. Less common causes are hypervitaminosis D, sarcoidosis, thyrotoxicosis, milk-alkali syndrome, adrenal insufficiency, and immobilization. With the possible exception of hypervitaminosis D, these latter disorders seldom require emergency lowering of serum calcium. A number of approaches are used to manage the hypercalcemic crisis.

Saline Diuresis

In hypercalcemia of sufficient severity to produce symptoms, rapid reduction of serum calcium is required. The first steps include rehydration with saline and diuresis with furosemide. Most patients presenting with severe hypercalcemia have a substantial component of prerenal azotemia owing to dehydration, which prevents the kidney from compensating for the rise in serum calcium by excreting more calcium in the urine. Therefore, the initial infusion of 500–1000 mL/h of saline to reverse the dehydration and restore urine flow can by itself substantially lower serum calcium. The addition of a loop diuretic such as furosemide (Chapter 15) not only enhances urine flow but also inhibits calcium reabsorption in

the ascending limb of the loop of Henle. Monitoring central venous pressure is important to forestall the development of congestive heart failure and pulmonary edema in predisposed subjects. In many subjects, saline diuresis will suffice to reduce serum calcium levels to a point at which more definitive diagnosis and treatment of the underlying condition can be achieved. If this is not the case or if more prolonged medical treatment of hypercalcemia is required, the following agents are available (discussed in order of preference).

Bisphosphonates

Etidronate, 7.5 mg/kg in 250–500 mL saline, infused over several hours each day for 3 days, has proved quite useful in treating hypercalcemia of malignancy. More recently, pamidronate, 60–90 mg in 500–750 mL saline, infused over 4–24 hours, has been approved for the same indication. This form of treatment is remarkably free of toxicity. The effects generally persist for weeks, but treatment can be repeated after a 7-day interval if necessary.

Calcitonin

Calcitonin has proved useful as ancillary treatment in a large number of patients. Calcitonin by itself seldom restores serum calcium to normal, and refractoriness frequently develops. However, its lack of toxicity permits frequent administration at high doses (200 MRC units or more). An effect on serum calcium is observed within 4–6 hours and lasts for 6–10 hours. Calcimar (salmon calcitonin) is available for parenteral administration only.

Gallium Nitrate

Gallium nitrate is approved by the Food and Drug Administration for the management of hypercalcemia of malignancy and is undergoing trials for the treatment of advanced Paget's disease. This drug acts by inhibiting bone resorption. At a dose of 200 mg/m^2 body surface area per day given as a continuous intravenous infusion in 5% dextrose for 5 days, gallium nitrate proved superior to calcitonin in reducing serum calcium in cancer patients. Because of potential nephrotoxicity, patients should be well-hydrated and have good renal output before starting the infusion.

Plicamycin (Mithramycin)

Because of its toxicity, plicamycin (mithramycin) is not the drug of first choice for the treatment of hypercalcemia. However, when other forms of therapy fail, 25–50 μg/kg given intravenously usually lowers serum calcium substantially within 24–48 hours. This effect can last for several days. This dose can be repeated as necessary. The most dangerous toxic effect is sudden thrombocytopenia followed by hemorrhage. Hepatic and renal toxicity can also occur. Hypocalcemia, nausea, and vomiting may limit therapy. Use of this drug must be accompanied by careful monitoring of platelet counts, liver and kidney function, and serum calcium levels.

Phosphate

Giving intravenous phosphate is probably the fastest and surest way to reduce serum calcium, but it is a hazardous procedure if not done properly. Intravenous phosphate should be used only after other methods of treatment (etidronate, calcitonin, saline diuresis with furosemide, and plicamycin) have failed to control symptomatic hypercalcemia. Phosphate must be given slowly (50 mmol or 1.5 g elemental phosphorus over 6–8 hours) and the patient switched to oral phosphate (1–2 g/d elemental phosphorus, as one of the salts indicated below) as soon as symptoms of hypercalcemia have cleared. The risks of intravenous phosphate therapy include sudden hypocalcemia, ectopic calcification, acute renal failure, and hypotension. Oral phosphate can also lead to ectopic calcification and renal failure if serum calcium and phosphate levels are not carefully monitored, but the risk is less and the time of onset much longer. Phosphate is available in oral and intravenous forms as the sodium or potassium salt. Amounts required to provide 1 g of elemental phosphorus are as follows:

Intravenous:
- In-Phos: 40 mL
- Hyper-Phos-K: 15 mL

Oral:
- Fleet Phospho-Soda: 6.2 mL
- Neutra-Phos: 300 mL

Glucocorticoids

Glucocorticoids have no clear role in the acute treatment of hypercalcemia. However, the chronic hypercalcemia of sarcoidosis, vitamin D intoxication, and certain cancers may respond within several days to glucocorticoid therapy. Prednisone in doses of 30–60 mg orally daily is generally used, though equivalent doses of other glucocorticoids are effective. The rationale for the use of glucocorticoids in these diseases differs, however. The hypercalcemia of sarcoidosis appears to be secondary to increased production of $1,25(OH)_2D$, possibly by the sarcoid tissue itself. Glucocorticoid therapy directed at the reduction of sarcoid tissue results in restoration of normal serum calcium and $1,25(OH)_2D$ levels. The treatment of hypervitaminosis D with glucocorticoids probably does not alter vitamin D metabolism significantly but is thought to reduce vitamin D-mediated intestinal calcium transport. An action of glucocorticoids to reduce vitamin D-mediated bone resorption has not been excluded, however. The effect of glucocorticoids on the hypercalcemia of cancer is probably twofold. The malignancies responding best to glucocorticoids (ie, multiple myeloma and related lymphoproliferative diseases) are sensitive to the lytic action

of glucocorticoids, so part of the effect may be related to decreased tumor mass and activity. Glucocorticoids have also been shown to inhibit the effectiveness of osteoclast-activating factor, a humoral substance or substances elaborated by multiple myeloma and related cancers that stimulate osteoclastic bone resorption. Other causes of hypercalcemia—particularly primary hyperparathyroidism—do not respond to glucocorticoid therapy.

This difference in response of the various forms of hypercalcemia to glucocorticoids forms the basis for the glucocorticoid suppression test, in which the response of serum calcium to a 10-day course of prednisone, 60 mg orally daily, helps differentiate the hypercalcemia of primary hyperparathyroidism from other causes such as sarcoidosis, vitamin D intoxication, and certain cancers. This test may be misleading and should not be used as a substitute for more specific tests for primary hyperparathyroidism such as serum immunoreactive PTH determinations.

HYPOCALCEMIA

The main features of hypocalcemia are neuromuscular—tetany, paresthesias, laryngospasm, muscle cramps, and convulsions. The major causes of hypocalcemia in the adult are hypoparathyroidism, vitamin D deficiency, renal failure, and malabsorption. Neonatal hypocalcemia is a common disorder that usually resolves without therapy. The roles of PTH, vitamin D, and calcitonin in the neonatal syndrome are under active investigation. Large infusions of citrated blood can produce hypocalcemia by the formation of citrate-calcium complexes. Calcium and vitamin D (or its metabolites) form the mainstay of treatment of hypocalcemia.

Calcium

A number of calcium preparations are available for intravenous, intramuscular, and oral use. Calcium gluceptate (0.9 meq calcium/mL), calcium gluconate (0.45 meq calcium/mL), and calcium chloride (0.68–1.36 meq calcium/mL) are available for intravenous therapy. Calcium gluconate is the preferred form because it is less irritating to veins. Oral preparations include calcium carbonate (40% calcium), calcium lactate (13% calcium), calcium phosphate (25% calcium), and calcium citrate (17% calcium). Calcium carbonate is often the preparation of choice because of its high percentage of calcium, ready availability (eg, Tums), low cost, and antacid properties. In achlorhydric patients, calcium carbonate should be given with meals to increase absorption. Combinations of vitamin D and calcium are available, but treatment must be tailored to the individual patient and individual disease, a flexibility lost by fixed-dosage combinations. Treatment of severe symptomatic hypocalcemia can be accomplished with slow infusion of 5–20 mL of 10% calcium gluconate. Rapid infusion can lead to cardiac arrhythmias. Less severe hypocalcemia is best treated with oral forms sufficient to provide approximately 400–800 mg of elemental calcium (1–2 g calcium carbonate per day). Dosage must be adjusted to avoid hypercalcemia and hypercalciuria.

Vitamin D

When rapidity of action is required, $1,25(OH)_2D_3$ (calcitriol), 0.25–1 μg daily, is the vitamin D metabolite of choice, since it is capable of raising serum calcium within 24–48 hours. Calcitriol also raises serum phosphate, though this action is usually not observed early in treatment. The combined effects of calcitriol and all other vitamin D metabolites and analogues on both calcium and phosphate make careful monitoring of these mineral levels especially important to avoid ectopic calcification secondary to an abnormally high serum calcium × phosphate product. Since the choice of the appropriate vitamin D metabolite or analogue for long-term treatment of hypocalcemia depends on the nature of the underlying disease, further discussion of vitamin D treatment will be found under the headings of the specific diseases.

HYPERPHOSPHATEMIA

Hyperphosphatemia is a frequent complication of renal failure but is also found in all types of hypoparathyroidism (idiopathic, surgical, and pseudo), vitamin D intoxication, and the rare syndrome of tumoral calcinosis. Emergency treatment of hyperphosphatemia is seldom necessary but can be achieved by dialysis or glucose and insulin infusions. In general, control of hyperphosphatemia involves restriction of dietary phosphate plus the use of phosphate binding gels such as $Al(OH)_3$-containing antacids and of calcium supplements. Because of their potential to induce aluminum-associated bone disease, aluminum-containing antacids should be used sparingly and only when other measures fail to control the hyperphosphatemia.

HYPOPHOSPHATEMIA

A variety of conditions are associated with hypophosphatemia, including primary hyperparathyroidism, vitamin D deficiency, idiopathic hypercalcemia, vitamin D-resistant rickets, various other forms of renal phosphate wasting (eg, Fanconi's syndrome), overzealous use of $Al(OH)_3$-containing antacids, and parenteral nutrition with inadequate phosphate content. Acute hypophosphatemia may lead to a reduction in the intracellular levels of high-energy organic phosphates (eg, ATP), interfere with normal hemoglobin-to-tissue oxygen transfer by decreasing red

active (as regards tumor resistance) with other alkylating agents; all appear to require biotransformation, which occurs by nonenzymatic decomposition, to derivatives with both alkylating and carbamoylating activities. The nitrosoureas are highly lipid-soluble and cross the blood-brain barrier, making them useful in the treatment of brain tumors. The nitrosoureas appear to function by cross-linking through alkylation of DNA. The drugs may be more effective against plateau phase cells than exponentially growing cells, although within a cycling cell population the drugs appear to slow cell progression through the DNA synthetic phase. After oral administration of CCNU or methyl-CCNU, plasma metabolites account for virtually all the administered drug, with peak plasma levels of metabolites appearing within 1–4 hours and prompt central nervous system appearance of 30–40% of the activity present in the plasma. While the initial plasma half-life is in the range of 6 hours, a second half-life is in the range of 1–2 days. Urinary excretion appears to be the major route of elimination from the body. One naturally occurring sugar-containing nitrosourea, streptozocin, is interesting because it has minimal bone marrow toxicity but is frequently effective in the treatment of insulin-secreting islet cell carcinoma of the pancreas and occasionally in non-Hodgkin lymphomas.

RELATED DRUGS PROBABLY ACTING AS ALKYLATING AGENTS

A variety of other compounds have mechanisms of action that involve alkylation. These include procarbazine, dacarbazine, altretamine (hexamethylmelamine), and cisplatin.

1. PROCARBAZINE

The oral agent procarbazine is a methylhydrazine derivative with chemotherapeutic activity (particularly in Hodgkin's disease). The drug is also leukemogenic and has teratogenic and mutagenic properties.

The mechanism of action of procarbazine is uncertain; however, the drug inhibits the synthesis of DNA, RNA, and protein; prolongs interphase; and produces chromosome breaks. Oxidative metabolism of this drug by microsomal enzymes generates azoprocarbazine and H_2O_2, which may be responsible for DNA strand scission. A variety of other metabolites of the drug are formed that may be cytotoxic. One metabolite is a monoamine oxidase (MAO) inhibitor, and adverse side effects can occur when procarbazine is given with other MAO inhibitors. In addition to predictable nausea, vomiting, and myelosuppression, hemolytic anemia, pulmonary reactions, and adverse responses with alcohol (disulfiram-like) have also been reported, as have skin rashes when procarbazine is given with phenytoin.

Procarbazine is often used in combination chemotherapy of Hodgkin's disease; however, its leukemogenic properties may eventually lead to its replacement with drugs that have lesser carcinogenic potential.

2. DACARBAZINE

Dacarbazine is a synthetic compound that functions as an alkylating agent following metabolic activation by liver microsomal enzymes by oxidative N-demethylation to the monomethyl derivative that spontaneously decomposes to 5-aminoimidazole-4-carboxamide, which is excreted in the urine, and diazomethane. The diazomethane generates a methyl carbonium ion that is believed to be the likely cytotoxic species. Dacarbazine is administered parenterally and is not schedule-dependent. It produces marked nausea, vomiting, and myelosuppression. Its major applications are in melanoma, Hodgkin's disease, and some soft tissue sarcomas. In the latter two tumors, its activity is potentiated by doxorubicin.

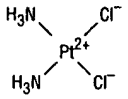
3. ALTRETAMINE (Hexamethylmelamine)

Altretamine is structurally similar to triethylenemelamine. It is relatively insoluble and available only in oral form. A related compound, pentamethylmelamine, which is a major metabolite of hexamethylmelamine, is more soluble and is now in clinical trial in an intravenous form. Both agents are rapidly biotransformed by demethylation, presumably to active intermediates. These agents cause nausea, vomiting, and central and peripheral nervous system neuropathies but relatively mild myelosuppression. Altretamine is useful in alkylating agent-resistant ovarian carcinoma.

4. CISPLATIN

Cisplatin (*cis*-diamminedichloroplatinum [II]) is an inorganic metal complex discovered by Rosenberg and his colleagues, who made the serendipitous observation that neutral platinum complexes inhibit division and induce filamentous growth of *Escherichia coli*. Many platinum analogues of this very important drug have been synthesized. While the precise mechanism of action of cisplatin is still undefined, it is thought to act analogously to alkylating agents. It kills cells in all stages of the cell cycle, inhibits DNA biosynthesis, and binds DNA through the formation of interstrand cross-links. The primary binding site is the N7 of guanine, but covalent interaction with ade-

nine and cytosine also occurs. The platinum complexes appear to synergize with certain other anticancer drugs. After intravenous administration, the major acute toxicity is nausea and vomiting. Cisplatin has relatively little effect on the bone marrow, but it can induce significant renal dysfunction and occasional acoustic nerve dysfunction. Hydration with saline infusion alone or with mannitol or other diuretics appears to minimize nephrotoxicity.



Cisplatin

Cisplatin has major antitumor activity in genitourinary cancers, particularly testicular, ovarian, and bladder cancer. Its use along with vinblastine and bleomycin has been a major advance in the development of curative therapy for nonseminomatous testicular cancers. A platinum analogue (carboplatin) with significantly less gastrointestinal and renal toxicity but with myelosuppressive toxicity provides a useful alternative to cisplatin.

Dosage & Toxicity of the Alkylating Agents

The alkylating agents are used in the treatment of a wide variety of hematologic and solid cancers, generally as components of combination chemotherapy. These are discussed under various specific tumors. Dosages and major toxicities are listed in Table 56–2.

Nausea and vomiting are almost universally reported with intravenously administered mechlorethamine, cyclophosphamide, and carmustine and occur with moderate frequency with oral cyclophosphamide.

The important toxic effect of therapeutic doses of virtually all the alkylating drugs is depression of bone marrow and subsequent leukopenia and thrombocytopenia. Severe infections and septicemia may result, with granulocytopenia below 600 PMNs/μL. Platelet depression below 40,000/μL may be accompanied by induced hemorrhagic phenomena. Cyclophosphamide may produce slight to severe alopecia in up to 30% of patients. It may also cause hemorrhagic cystitis. The cystitis can often be averted with adequate hydration.

The hematopoietic effects of toxic doses of alkylating drugs are treated by discontinuing the agent. Red cell and platelet transfusions and antibiotics to con-

Table 56–2. Polyfunctional alkylating agents and probable alkylating agents: Dosages and toxicity.

Alkylating Agent	Single-Agent Dosage	Acute Toxicity	Delayed Toxicity
Nitrogen mustard (HN2, mechlorethamine, Mustargen)	0.4 mg/kg IV in single or divided doses	Nausea and vomiting	Moderate depression of peripheral blood count. Excessive doses produce severe bone marrow depression with leukopenia, thrombocytopenia, and bleeding. Alopecia and hemorrhagic cystitis occasionally occur with cyclophosphamide. Cystitis can be prevented with adequate hydration. Busulfan is associated with skin pigmentation, pulmonary fibrosis, and adrenal insufficiency.
Chlorambucil (Leukeran)	0.1–0.2 mg/kg/d orally, 6–12 mg/d	None	
Cyclophosphamide (Cytoxan)	3.5–5 mg/kg/d orally for 10 days; 1 g/m² IV as single dose	Nausea and vomiting	
Melphalan (Alkeran)	0.25 mg/kg/d orally for 4 days every 4–6 weeks	None	
Thiotepa (triethylenethiophosphoramide)	0.2 mg/kg IV for 5 days	None	
Busulfan (Myleran)	2–8 mg/d orally; 150–250 mg/course	None	
Carmustine (BCNU)	200 mg/m² IV every 6 weeks	Nausea and vomiting	Leukopenia and thrombocytopenia; rarely hepatitis.
Lomustine (CCNU)	130 mg/m² orally every 6 weeks	Nausea and vomiting	
Semustine (methyl-CCNU)	150 mg/m² orally every 6 weeks	Nausea and vomiting	
Altretamine (hexamethylmelamine)	10 mg/kg/d for 21 days	Nausea and vomiting	Leukopenia, thrombocytopenia, and peripheral neuropathy.
Procarbazine (Matulane)	50–200 mg/d orally	Nausea and vomiting	Bone marrow depression, central nervous system depression.
Dacarbazine	300 mg/m² daily IV for 5 days	Nausea and vomiting	Bone marrow depression.
Cisplatin (Platinol)	20 mg/m²/d IV for 5 days or 50–70 mg/m² as single dose every 3 weeks	Nausea and vomiting	Renal dysfunction. Acoustic nerve dysfunction.
Carboplatin (Paraplatin)	360 mg/m² IV every 4 weeks	Nausea and vomiting	Leukopenia and thrombocytopenia. Rarely neuropathy or hepatic dysfunction.

trol infections are employed as needed until the marrow has regenerated.

ANTIMETABOLITES (Structural Analogues)

The development of drugs with actions on intermediary metabolism of proliferating cells has been important both clinically and conceptually. While biochemical properties unique to all cancer cells have yet to be discovered, neoplastic cells do have a number of quantitative differences in metabolism from normal cells that render them more susceptible to a number of antimetabolites or structural analogues. Many of these agents have been rationally designed and synthesized, based on knowledge of cellular processes, although a few have been discovered as antibiotics.

Mechanisms of Action

The biochemical pathways that have thus far proved to be most exploitable with antimetabolites have been those relating to nucleotide and nucleic acid synthesis. In a number of instances, when an enzyme is known to have a major effect on pathways leading to cell replication, inhibitors of the reaction it catalyzes have proved to be useful anticancer drugs.

These drugs and their doses and toxicities are shown in Table 56–3. The principal drugs are discussed below.

METHOTREXATE

Methotrexate (MTX) is a folic acid antagonist that binds to the active catalytic site of dihydrofolate reductase (DHFR), interfering with the synthesis of the reduced form that accepts one-carbon units. Lack of this cofactor interrupts the synthesis of thymidylate, purine nucleotides, and the amino acids serine and methionine, thereby interfering with the formation of DNA, RNA, and protein. The enzyme binds methotrexate extremely tightly, and at pH 6.0 virtually no dissociation of the enzyme-inhibitor complex occurs (inhibition constant about 1 nmol/L). At physiologic pH, reversible competitive kinetics occur (inhibition constant about 1 μmol/L). Intracellular formation of polyglutamate derivatives appears to be important in the therapeutic action of methotrexate (Jolivet, 1983). The polyglutamates of methotrexate are retained by cells longer than methotrexate and have increased inhibitory effects on enzymes involved in folate metabolism, making them important determinants in the duration of action of methotrexate.

Drug Resistance

Tumor cell resistance to methotrexate has been attributed to (1) decreased drug transport, (2) altered DHFR with lower affinity for methotrexate, (3) decreased polyglutamate formation, and (4) synthesis of

Table 56–3 Structural analogues. Dosages and toxicity.

Chemotherapeutic Agent	Single Agent Dosage	Delayed Toxicity[1]
Azacitidine[2]	200 mg/m²/d IV for 5 days	Nausea and vomiting, diarrhea, fever, hypotension, prolonged marrow hypoplasia
Cladribine (Leustatin)	0.09 mg/kg/d for 9 days by IV infusion	Severe myelosuppression for about 2 weeks after therapy
Cytarabine (ara-C, Cytosar-U)	100 mg/m²/d for 5–10 days, either by continuous IV infusion or SC every 8 hours	Nausea and vomiting, bone marrow depression, megaloblastosis, leukopenia, thrombocytopenia
Fludarabine (Fludara)	25 mg/m²/d for 5 days every 28 days (administer IV over 30 minutes)	Myelosuppression. ***Note:*** High doses can cause serious neurotoxicity
Fluorouracil (5-FU, Adrucil)	15 mg/kg/d IV for 5 days by 24-hour infusion; 15 mg/kg weekly IV	Nausea, oral and gastrointestinal ulceration, bone marrow depression
Mercaptopurine (6-MP, Purinethol)	2.5 mg/kg/d orally	Usually well tolerated. Larger dosages may cause bone marrow depression
Methotrexate (amethopterin, MTX)	2.5–5 mg/d orally (Rheumatrex); 10 mg intrathecally (Folex) once or twice weekly	Oral and gastrointestinal tract ulceration, bone marrow depression, leukopenia, thrombocytopenia
Thioguanine (6-TG)	2 mg/kg/d orally	Usually well tolerated. Larger dosages may cause bone marrow depression
Supportive agent with all drugs		
Allopurinol (Zyloprim)	300–800 mg/d orally for prevention or relief of hyperuricemia	Usually none. Enhances effects and toxicity of mercaptopurine when used in combination

[1]These drugs do not cause acute toxicity.
[2]For use only in approved treatment protocols.

cardium by doxorubicin. This is rarely seen at doxorubicin dosages below 500 mg/m^2. Use of lower weekly doses or continuous infusions of doxorubicin that avoid high peak plasma concentrations appear to reduce the frequency of cardiac toxicity as compared to intermittent (every 3–4 weeks) higher dosage schedules.

A second toxicity of doxorubicin and daunorubicin is the almost universal occurrence of severe or total alopecia at standard dosages.

DACTINOMYCIN

Dactinomycin is an antitumor antibiotic isolated from a *Streptomyces.*

Mechanism of Action & Pharmacokinetics

Dactinomycin binds tightly to double-stranded DNA through intercalation between adjacent guanine-cytosine base pairs. Dactinomycin inhibits all forms of DNA-dependent RNA synthesis, with ribosomal RNA formation being most sensitive to drug action. DNA replication is much less reduced, but protein synthesis is blocked in affected cells. The degree of responsiveness to dactinomycin appears to be dependent on the cellular capability for accumulation and retention of the antibiotic.

Approximately half of the intravenous dose of dactinomycin (Table 56–4) remains unmetabolized and is excreted in the bile; a small amount is lost by urinary excretion. The plasma half-life is short. Because the drug is irritating to tissues, it is usually administered with caution to avoid extravasation and with "flushing" with normal saline to wash out the vein.

Clinical Uses

Dactinomycin is used in combination with surgery and vincristine (with or without radiotherapy) in the adjuvant treatment of Wilms' tumor. It is also used along with methotrexate to provide potentially curative treatment for patients with localized or disseminated gestational choriocarcinoma.

Adverse Reactions

Bone marrow depression, the major dose-limiting toxicity of this agent, is usually evident within 7–10 days. All blood elements are affected, but platelets and leukocytes are affected most profoundly and severe thrombocytopenia sometimes occurs. Nausea and vomiting, diarrhea, oral ulcers, and skin eruptions may also be noted. The agent is also immunosuppressive, and patients receiving this drug should not receive live virus vaccines. Alopecia and various skin abnormalities occur occasionally. As with anthracyclines, dactinomycin can interact with radiation, producing a "radiation recall" skin abnormality associated with inflammation at sites of prior radiation therapy.

PLICAMYCIN

Plicamycin (formerly mithramycin) is one of the chromomycin antibiotics isolated from *Streptomyces plicatus.* Plicamycin's mechanism of action appears to involve its binding to DNA, possibly through an antibiotic-Mg^{2+} complex; this interaction interrupts DNA-directed RNA synthesis. In addition, the drug causes plasma calcium levels to decrease, apparently through an action on osteoclasts that is independent of its action on tumor cells (useful in hypercalcemia; see Chapter 41). The drug has some usefulness in testicular cancers refractory to standard treatment, but it is of more use in reversing severe hypercalcemia associated with malignant disease.

Toxic effects of plicamycin include nausea and vomiting, thrombocytopenia, leukopenia, hypocalcemia, bleeding disorders, and liver toxicity. Aside from its use in management of hypercalcemia, plicamycin currently has few other indications.

MITOMYCIN

Mitomycin (mitomycin C) is an antibiotic isolated from *Streptomyces caespitosus.* It contains quinone, carbamate, and aziridine groups, all of which may contribute to its activity. The drug is a "bioreductive" alkylating agent that undergoes metabolic reductive activation through an enzyme-mediated reduction to generate an alkylating agent that cross-links DNA. Hypoxic tumor stem cells of solid tumors exist in an environment conducive to reductive reactions and are more sensitive to the cytotoxic actions of mitomycin than normal and oxygenated tumor cells. Mitomycin is thought to be a CCNS alkylating agent. While this agent is one of the more toxic drugs available for clinical use, it is the best available drug for use as an adjuvant to x-irradiation to attack hypoxic tumor cells. It is being used increasingly in combination chemotherapy (with bleomycin and vincristine) for squamous cell carcinoma of the cervix and for adenocarcinomas of the stomach, pancreas, and lung (along with doxorubicin and fluorouracil). The drug also has some usefulness as a second-line agent for metastatic colon cancer. A special application of mitomycin has been in topical intravesical treatment of small bladder papillomas. Instillations of the agent in distilled water are usually held in the bladder for 3 hours, and the procedure is repeated over a course of weeks. Very little of the agent is absorbed systemically, and it can be quite effective at reducing the frequency of such bladder tumors.

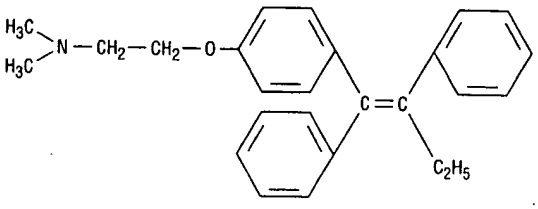
and estrogens will cause masculinization and feminization, respectively. Extended use of the adrenocortical steroids may result in hypertension, diabetes, increased susceptibility to infection, and the development of cushingoid appearance ("moon facies"; see Chapter 38 and Table 56–5).

ESTROGEN & ANDROGEN INHIBITORS

The estrogen inhibitor tamoxifen has proved to be extremely useful for the treatment of breast cancer. It also has activity against progesterone-resistant endometrial cancer. Tamoxifen is also currently undergoing clinical trial as a chemopreventive agent in women at high risk for breast cancer. Tamoxifen functions as a competitive partial agonist-inhibitor of estrogen and binds to the estrogen receptors of estrogen-sensitive tissues and tumors. However, tamoxifen has a tenfold lower affinity constant for ER than does estradiol, indicating the importance of ablation of endogenous estrogen for optimal antiestrogen effect.

Excellent plasma levels of tamoxifen are obtained after oral administration, and the agent has a much longer biologic half-life than estradiol. The usual dosage is 10 mg twice daily (Table 56–5), although doses up to 100 mg/m^2 have been administered without major toxicity. Because it may take several weeks to achieve a steady-state level of the active metabolite (monohydroxytamoxifen) with the dosage of 20 mg/d, it is advisable to give an 80 mg "loading course" on the first day to achieve good blood levels rapidly.

Adverse effects in the usual dose range are quite mild. Hot flashes are the most frequent effect. Nausea is observed occasionally, as is fluid retention. Occasional "flares" of breast cancer are observed that usually subside with continued therapy.



Tamoxifen

In advanced breast cancer, clinical improvement is observed in 40–50% of patients who receive tamoxifen. Patients who show objective benefit with treatment are largely those who (1) lack endogenous estrogens (oophorectomy or postmenopausal state) and (2) have breast cancers in which the cytoplasmic ER or PR protein is demonstrable. Tamoxifen has also been effective at prolonging survival when used as surgical adjuvant therapy for postmenopausal women with ER-positive breast cancer. In addition to its direct antiestrogen effects on tumor cells, tamoxifen also suppresses serum levels of insulin-like growth factor-1 and up-regulates local production of transforming growth factor-beta (TGFβ). These endocrine effects of tamoxifen may account for some of its antitumor actions in other tumor types such as ovarian cancer and melanoma.

An antiandrogen, flutamide, has been approved by the FDA for use in the treatment of prostate cancer. It is used to antagonize residual androgenic effects after orchiectomy or with leuprolide (see below). Finasteride, a nonsteroidal inhibitor of 5α-testosterone reductase (Chapter 39), the enzyme that converts testosterone to dihydrotestosterone, is investigational in the treatment of cancer. It is promising because it may prevent androgen effects in prostatic tumor tissue without suppressing androgen production or effects in other tissues.

GONADOTROPIN-RELEASING HORMONE AGONISTS

Leuprolide acetate and goserelin are synthetic peptide analogues of naturally occurring gonadotropin-releasing hormone (GnRH, LHRH). They are described in Chapters 36 and 39. These analogues are more potent than the natural hormone and function as GnRH agonists, with paradoxic results, when given continuously (or as a depot preparation), on the pituitary—initial stimulation followed by inhibition of the release of follicle-stimulating hormone and luteinizing hormone. This results in reduced testicular androgen synthesis. The latter effect underlies the efficacy of these agents in the treatment of metastatic carcinoma of the prostate.

Leuprolide has been directly compared with standard endocrine therapy of prostatic cancer with diethylstilbestrol (DES). Suppression of androgen synthesis and reductions in serum prostatic acid phosphatase (a marker of metastatic tumor burden) are comparable with both leuprolide and DES. However, painful gynecomastia, nausea, vomiting, edema, and thromboembolism occur significantly less frequently with leuprolide than with DES. Leuprolide and goserelin are more expensive than DES, but that is more than offset by the overall cost of complications of DES or the surgical and hospitalization costs associated with surgical orchiectomy. Long-acting depot formulations of both leuprolide and goserelin are administered once monthly. The endocrine effects of these agents may also prove useful in the management of hormone receptor-positive breast cancer, but this application remains to be established.

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fficient to relieve moderate-to-severe pain in 70% of patients. How-er, doses need to be tailored to the patient based on individual sen-ivity to the drug and the pain-sparing needs of the individual. In-ıvenous administration may be indicated for severe pain, using ıher continuous infusion or intermittent dosing. Patient-controlled algesia (PCA), in which the patient has limited control over the se and/or dosing interval, has proven valuable and has gained wide ceptance in the control of postoperative pain. Morphine also is used idurally and intrathecally in selected situations (Foley, 1993); a eservative-free morphine sulfate sterile solution (INFUMORPH) is ailable for use in continuous microinfusion devices for intraspinal ministration.

Morphine is available orally in standard tablets and controlled-ease preparations. Due to first-pass metabolism, morphine is two- six-fold less potent orally than parenterally. This is important to nember when converting a patient from parenteral to oral med-tion. There is wide variability in the first-pass metabolism, and the se should be titrated to the patient's needs. In children who weigh s than 50 kg, morphine can be given at 0.1 mg/kg every 3 to 4 urs parenterally or at 0.3 mg/kg orally.

Codeine is widely used orally due to its high oral/parenteral po-cy ratio. Orally, codeine at 30 mg is approximately equianalgesic to 5 to 600 mg of aspirin. Combinations of codeine with aspirin or ac-minophen usually provide additive actions, and at these doses anal-ic efficacy can exceed that of 60 mg of codeine (*see* Beaver, 1988).

Many drugs can be used instead of either morphine or codeine, shown in Table 23–6. Oxycodone, with its high oral/parenteral po-cy ratio, is widely used in combination with aspirin (PERCODAN) acetaminophen (PERCOCET), although it is available alone (ROXI-DONE).

Heroin (diacetylmorphine) is not available for therapeutic use he United States, although it has been used in the United King-n. Given intramuscularly, it is approximately twice as potent as rphine. Pharmacologically, heroin is very similar to morphine and s not appear to have any unique therapeutic advantages over the ilable opioids (Sawynok, 1986; Kaiko *et al.*, 1981).

It also may be helpful to employ other agents (adjuvants) that ance opioid analgesia and that may add beneficial effects of their ı. For example, the combination of an opioid with a small dose mphetamine may augment analgesia while reducing the sedative cts. Certain antidepressants, such as amitriptyline and des-mine also may enhance opioid analgesia, and they may have anal-c actions in some types of neuropathic (deafferentation) pain (*see* Quay, 1988). Other potentially useful adjuvants include certain histamines, anticonvulsants such as carbamazepine and pheny-, and glucocorticoids.

When the pain is associated with biliary spasm, meperidine or of the agonist/antagonist opioids may produce less increase in spasm than will an equianalgesic dose of morphine or a similar ıt. When the pain is likely to be of short duration (*e.g.*, diagnos-rocedures, cystoscopy, orthopedic manipulation), a drug with a ter duration of action, such as alfentanil, might be preferable to phine or oxycodone.

of Terminal Illness and Cancer Pain. Opioids are not indi-d in all cases of terminal illness, but the analgesia, tranquility, even the euphoria afforded by the use of opioids can make the days far less distressing for the patient and family. Although ical dependence and tolerance may develop, this possibility ld not in any way prevent physicians from fulfilling their pri-obligation to ease the patient's discomfort. The physician should wait until the pain becomes agonizing; *no patient should ever wish for death because of a physician's reluctance to use adequate amounts of effective opioids.* This may sometimes entail the regular use of opioid analgesics in substantial doses (*see* below). Such patients, while they may be physically dependent, are not "addicts" even though they may need large doses on a regular basis. Physical dependence alone does not fulfill the criteria for drug addiction (*see* Chapter 24).

Most clinicians who are experienced in the management of chronic pain associated with malignant disease or terminal illness recommend that opioids be administered at sufficiently short, fixed intervals so that pain is continually under control and patients do not dread its return (Foley, 1993). Less drug is needed to prevent the recurrence of pain than to relieve it. Morphine remains the opioid of choice in most of these situations, and the route and dose should be adjusted to the needs of the individual patient. Many clinicians find that oral morphine is adequate in most situations. Sustained-release preparations of oral morphine are now available that can be administered at 8- to 12-hour intervals. Superior control of pain often can be achieved with fewer side effects using the same daily dose; a decrease in the fluctuation of plasma concentrations of morphine may be partially responsible.

Constipation is an exceedingly common problem when opioids are used, and the use of stool softeners and laxatives should be initiated early. Amphetamines have demonstrable mood-elevating and analgesic effects and enhance opioid-induced analgesia. However, not all terminal patients require the euphoriant effects of amphetamine, and some experience side effects, such as anorexia. Controlled studies demonstrate no superiority of oral heroin over oral morphine. Similarly, after adjustment is made for potency, parenteral heroin is not superior to morphine in terms of analgesia, effects on mood, or side effects (*see* Sawynok, 1986). Although tolerance does develop to oral opioids, many patients obtain relief from the same dosage for weeks or months.

When opioids and other analgesics are no longer satisfactory, nerve block, chordotomy, or other types of neurosurgical intervention such as neurostimulation may be required if the nature of the lesion permits. Epidural or intrathecal administration of opioids may be useful when administration of opioids by usual routes no longer yields adequate relief of pain. This technique has been used with ambulatory patients over periods of weeks or months (*see* Gustafsson and Wiesenfeld-Hallin, 1988). Moreover, portable devices have been developed that permit the patient to control the parenteral administration of an opioid while remaining ambulatory (Kerr *et al.*, 1988). These devices use a pump that infuses the drug from a reservoir at a rate that can be tailored to the needs of the patient, and they include mechanisms to limit dosage and/or allow the patient to self-administer an additional "rescue" dose if there is a transient change in the intensity of pain.

Postoperative Pain. When pain is not too severe, oral codeine or oxycodone combined with nonsteroidal antiinflammatory agents often provides adequate analgesia without the side effects associated with the use of usual doses of morphine. When pain is more severe, opioid analgesics are used in the immediate postoperative period. However, if used excessively, they may prevent the early recognition of complications, decrease the effectiveness of coughing, decrease respiratory ventilation, predispose to pneumonitis, reduce bowel motility, and cause urinary retention. Used properly, however, the reduction of pain may increase the patient's ability to breathe deeply, cooperate with respiratory therapy procedures, cough voluntarily, and ambulate. The use of fixed doses given "as needed" without consideration of individual requirements often leads to unnecessary suffering. Moreover,

IDW

TRANSMITTAL LETTER (General - Patent Pending)	Docket No. 115808-457

In Re Application Of: **Nongnuch Inpanbutr**

AF

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
09/804,111	March 12, 2001	Hui, San Ming	29157	1617	8670

Title: **METHOD AND PRODUCT FOR TREATING CANCER IN PETS**

COMMISSIONER FOR PATENTS:

Transmitted herewith is:

Transmittal of Appeal Brief (duplicate); Appeal Brief (22 pgs. in triplicate); Claims Appendix (5 pgs,); Evidence Appendix (1 pg.); Exhibits A-E; check in the amount of $500.00; return receipt postcard

in the above identified application.

- ☐ No additional fee is required.
- ☒ A check in the amount of **$500.00** is attached.
- ☒ The Director is hereby authorized to charge and credit Deposit Account No. **02-1818** as described below.
  - ☐ Charge the amount of
  - ☒ Credit any overpayment.
  - ☒ Charge any additional fee required.
- ☐ Payment by credit card. Form PTO-2038 is attached.

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Dated: **April 13, 2004**

Signature

Robert M. Barrett (Reg. No. 30,142)
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Fax: 312/827-8185

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04/13/05
(Date)

Signature of Person Mailing Correspondence

Heather Foster

Typed or Printed Name of Person Mailing Correspondence

cc:

TRANSMITTAL OF APPEAL BRIEF (Large Entity)	Docket No. 115808-457

In Re Application Of: **Nongnuch Inpanbutr**

Application No.	Filing Date	Examiner	Customer No.	Group Art Unit	Confirmation No.
09/804,111	March 12, 2001	Hui, San Ming	29157	1617	8670

Invention: **METHOD AND PRODUCT FOR TREATING CANCER IN PETS**

COMMISSIONER FOR PATENTS:

Transmitted herewith in triplicate is the Appeal Brief in this application, with respect to the Notice of Appeal filed on **February 24, 2005**

The fee for filing this Appeal Brief is: **$500.00**

☒ A check in the amount of the fee is enclosed.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account.

☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **02-1818**

☐ Payment by credit card. Form PTO-2038 is attached.

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